THE ROLE OF CUES AND THE HIPPOCAMPUS IN HOME BASE BEHAVIOUR

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DEDICATION

For my parents who instilled curiosity in me.

Especially my father,

Who guided me through my first experiment involving *Rhizopus nigricans,*

And has sent me looking for answers ever since.

ABSTRACT

The thesis examines the ability of animals to construct a home base. The home base is a point in space where animals rear, groom, and circle and is a primary element in organized spatial behaviour (Eilam and Golani 1989). Once animals establish a home base, they make outward trips and stops, and after a series of trips and stops they return again to the home base. The home base behaviour of animals acts as a platform for asking questions about the cognitive organization of an environment. The thesis describes five main findings. Control and hippocampectomized animals use (1) proximal and (2) distal cues to form a home base and organize their behaviour. (3) Control and olfactory bulbectomized animals form home bases in the dark where as hippocampectomized animals are impaired suggesting self-movement but not olfactory cues play a role in home base behaviour. A final set of experiments demonstrated that control and hippocampectomized animals learn the position of (4) proximal and (5) distal cues so that in the cue's absence, animals still form a home base at that position. The demonstration that a central feature of exploratory behaviour, establishing a home base, is preserved in hippocampectomized rats in relation to proximal, distal, and conditioned visual cues - reveals that exploratory behaviour remains organized after hippocampal lesions. The inability of hippocampectomized rats to form a virtual home base in the absence of visual cues is discussed in relation to the idea that the hippocampus contributes to inertial behaviour that may be dependent upon self-movement cues.

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CHAPTER 1

INTRODUCTION

7. *Physics and Cognitive Space*

i. Physics and Space

An understanding of time and space is undoubtedly one of the greatest problems that science has tried to solve (Hawkings & Penrose, 1995). The study of time and space has led to complete paradigm shifts in many of the different disciplines of science (Kuhn, 1970). From the impetus of these new theories come philosophies that advance science as a whole. A theoretical revolution in physics during the early part of the 20th century has imparted such advancements on the study of space and specifically cognitive space and memory. The idea that "space and time coordinates are only the elements of a language that is used by an observer to describe his environment" Sachs (1996) shows that this effect has spread into the domain of Neuroscience (Best & White, 1998).

Changes in the thinking of Neuroscientists can be seen from the implications of simply being aware of our space to research on the quantum physics of consciousness (McCrone, 2003). Scientists have readily explored space as a cognitive construct even though the boundaries and a complete understanding of physical space are unknown. The mechanism by which the brain constructs space, to the organization of this construction, and finally the physical evidence of spatial-temporal processing all provide insight into the human sense of space and reality (Best and White, 1998). The temporal recollection of space also gives insight into the mechanisms of memory.

ii. Tolman

The modern era of investigation into the spatial functions of the brain began with Tolman's (1948) interpretation of his sunburst maze experiment. Tolman's research was directed toward understanding the relationship between space and the brain. In contrast with the stimulis-response theorists of his time, Tolman proposed a cognitive theory for how organisms process and move through space.

In his seminal experiment Tolman used two variations of a sunburst maze (Fig. 1.1). In the first version of the maze, the animal's task was to reach a goal containing a food reward by following the only available L-shaped alley (Fig. 1.1a). Once animals readily reached the goal, the second version of the maze was used. In the second version a rat could chose from a variety of paths, one of which was a short-cut (Fig. 1.1b). The short-cut offered a direct path to the goal rather than the circuitous path that the rats had been trained on. Tolman found that the rats did select the short-cut path.

Tolman's interpretation of the result from the experiment was that animals form a cognitive map of their environment during the exposure to the first version of the maze. Using this map, and the configurations of cues external to the maze, the animal was able to take a short-cut to the goal. Tolman argues that rats do not just memorize the individual alleys but instead form ".. .a wider comprehensive map to the effect that the food was located in such and such direction in the room" (p.204).

Although Tolman's ideas about the existence of a cognitive map in rats helped gain impetus against stimulus-response (S-R) theorists of the time, many people

Figure 1.1. Diagrammatic representation of the maze variations used by Tolman. Diagram a. shows the first version in which rats were trained to travel down the L-shaped alley and retrieve a food reward. In the second version (diagram b.) rats were allowed to choose from a variety of paths, including one short-cut path.

A.

B.

started to shift their ideas towards a S-R theory of learning. The S-R theorists felt that many aspects of what was called the place behaviour could be readily accounted for by a response to a particular environmental stimuli (Restle, 1957).

One of the scientists who kept with the view that animals had an internal cognitive map of their environment was Donald Hebb (Hebb, 1961). Hebb reported what he called "position habits" and how rats interpreted spatial information as proof that animals were using their internal representations of the environment to guide spatial behaviours. Hebb's theories have influenced modern ideas about the brain through his writing and his students, including John O'Keefe and Lynn Nadel (Best & White, 1999).

//. *Anatomical Space*

i. Place Cells

Tolman's idea of a cognitive map was taken up by O'Keefe and Dostrovsky (1971) who postulated that the hippocampus is involved in spatial mapping. O'Keefe and Dostrovsky fitted rats with tiny microdrives that positioned microelectrodes into the hippocampus, so that the activity of neurons in the area could be recorded as the animals moved around. The behaviour of the freely moving animals was recorded while they were on a 24 cm x 36 cm raised platform. The sides of the platform were enclosed with a white curtain. The experiment recorded signals during sniffing, walking, eating, drinking, grooming and sleeping behaviours. The basic firing patterns of the cells found were recorded for 15 to 30 minutes.

Out of 76 cells that were found, the properties of 8 cells are described in the 1971 paper of O'Keefe and Dostrovsky. The property of the 8 cells of interest was that they "responded solely or maximally when the rat was situated in a particular part of the testing platform, facing in a particular direction" (p. 172). Despite trying to get these cells to fire via different stimuli, the only method for eliciting spike patterns was to place the animal in the same location facing in the appropriate direction.

O'Keefe and Dostrovsky (1971) tried to isolate the cues that were responsible for the firing of the eight particular cells. They tried to change sounds and olfactory cues in the environment. Only after extreme radial changes to several visual cues in the environment was the cell firing changed.

On the basis of this finding they suggested that a spatial map was formed and maintained by the hippocampus. Their idea was that the spatial reference map was created by correlating cues in the environment to the animal's position in space. They further proposed that if cues were random, or if the animals were deprived of a map, exploration would be initiated so that an animal could establish a new map. Finally they proposed that the loss of the spatial reference map is what is basic to the deficits seen in hippocampectomized rats.

ii. Hippocampal Anatomy

The hippocampus is both one of the largest and most interconnected structures in the rat brain (Amaral & Witter, 1989). The hippocampus forms the central axis of the limbic system and is often called the archecortex. Although usually thought of as a subcortical structure, the hippocampus has been shown to be a cortical infolding, and

therefore not a true subcortical structure (Amaral & Witter, 1989). In the rat brain, studies have shown that the hippocampus is only slightly smaller than the entirety of the cortex. The total surface area of the cortex is around 1.5 cm^2 where as the hippocampal formation is around 1.2 cm^2 (Swanson & Cowan, 1977).

The large size of the hippocampus and the high degree of connectivity has made it one of the earliest and most studied structures in the brain. Ramon Y Cajal (Cajal, 1911) and Rafael Lorente de No (Lorente De No, 1933) used a silver impregnation technique to view the dendrites and spines of hippocampal cells. Since the work of Cajal and Lorente de No, others have employed new techniques and have further described the structure and connectivity of the hippocampus (Blackstad, 1969).

When talking about the hippocampus and its surrounding structures, the term hippocampal formation is often used. The hippocampal formation is comprised of six distinct regions (Amaral & Witter, 1989). The hippocampus proper, the dentate gyrus, presubiculum, subiculum, parasubiculum, and the entorhinal cortex are the six respective regions (Fig. 1.2).

The hippocampus proper can also be further divided into its three constituent areas; the CA1, CA2, and CA3 (Fig. 1.3). The regions are distinct from one another because of differences in cell structures or intrinsic connectivity. The layers appear as two interlocking "Cs" and each contain specific cell types. The outermost C is called Ammons horn (Cornu Ammonis) and contains the CA1-CA3. The innermost C contains the dentate gyrus. The Ammons horn is cytoarchitecturially differentiated because it consists mainly of pyramidal cells. The dentate gyrus is also easily differentiated from other hippocampal areas because it mainly consists of granule cells.

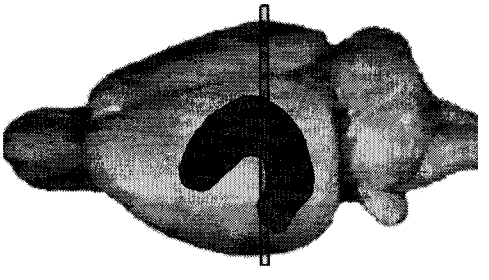
Figure 1.2. Diagram of the six regions of the Hippocampal Formation. The hippocampus proper (shown in purple), the dentate gyrus (shown in yellow), presubiculum, subiculum, parasubiculum (or subicular region; shown in green), and the entorhinal cortex (shown in blue) are the six respective regions.

Hippocampus Proper

Entorhinal Cortex

Subicular Region
(para-, pre-, subiculum)

Dentate Gyrus



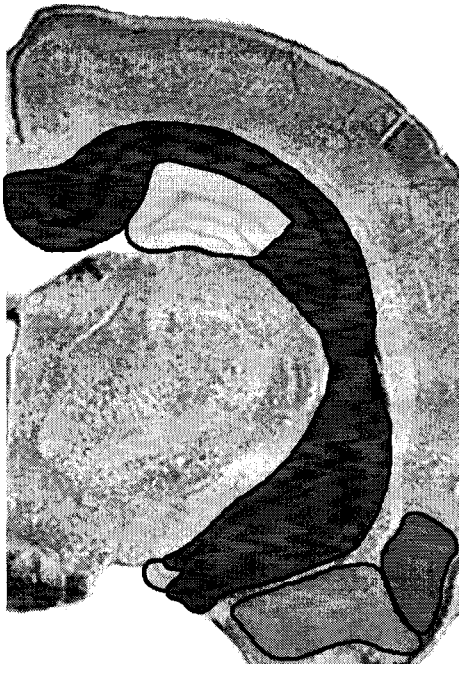
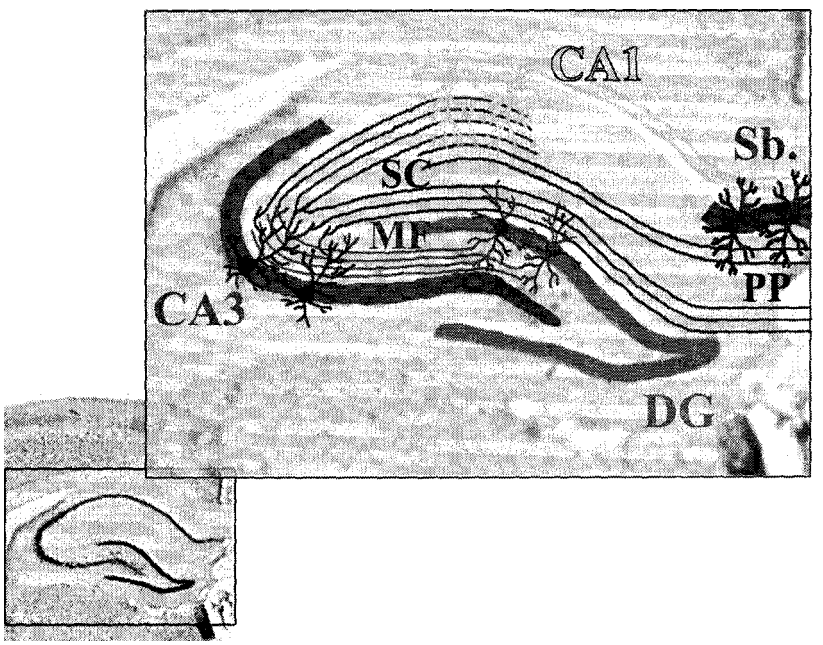


Figure 1.3. Diagram of the Trisynaptic Pathway. The hippocampal formation is composed of a loop-like network of connectivity. The loop begins with the Perforant Path (PP; shown in black), which is composed of inputs from the Entorhinal Cortex. These inputs synapse onto pyramidal cells in the Dentate Gyrus (DG; shown in light green) and CA3 field (shown in light blue). CA3 neurons also receive input via the Mossy Fibers (MF; shown in dark green) from the DG. CA3 projects to CAl (shown in yellow) via the Schaffer Collateral pathway (SC; shown in dark blue). CAl neurons also receive direct input from the Perforant Path, and send the major Hippocampal output to the Subiculum. Projections from the Subiculum to the Entorhinal Cortex complete the loop.



A-

A

Trisynaptic Pathway

The trisynaptic pathway (loop or circuit) is the primary path for information to flow through the hippocampus (Kelso, Ganong, & Brown, 1986). The major connections in the first stage of the path are in the entorhinal cortex, which project to the dentate granule cells through the perforant path (Amaral & Witter, 1989; Fig. 1.3). The granule cells synapse on CA3 pyramidal cells through mossy fibers. CA3 pyramidal neurons project to CA1 pyramidals by Schaffer collaterals, and finally the CA1 cells branch back to the entorhinal cortex via the subiculum.

Although the trisynaptic pathway is usually composed of the aforementioned paths, all of the hippocampus is also modified and modulated by other pathways. For instance, the collateral connections between CA3 neurons in the perforant path synapses onto CA1 and CA3 cells to act as inhibitory modulations of information coming from both directions of the path (Kelso et al., 1986). These and other associative type pathways act as a possible circuitry from memories and enable mechanism associated with learning.

Long term potentiation (LTP) has been demonstrated in the hippocampus at the synapses of recurrent collaterals in CA3 (Bliss & Lomo, 1973). LTP provides a mechanism for the strengthening of synapses between neurons that fire together.

iii. Cognitive Maps

Tolman's idea of a cognitive map was further elaborated upon O'Keefe and Nadel (1978) in their book "The hippocampus as a cognitive map." In their book, they posited a theory that hinged on three points. Firstly, the hippocampus was an important part of the

brain for processing spatial information; secondly that there is a cognitive representation of space and thirdly, memory is context dependent.

O'Keefe and Nadel (1978) postulated that there are two strategies for navigating. Both strategies are effective means for reaching a goal but are methodologically quite different from one another. They labeled these navigation strategies as taxon (route) and locale (map) navigation (Table 1.1).

Taxon

Taxon strategies are defined by O'Keefe and Nadel (1978) as "lists of guidance and orientation" (p. 96). The taxon hypothesis states that guidance behaviours of the environment act like cues and orientation properties are the animal's response to these cues. The animal acts in a goal directed fashion towards a taxon cue in the environment, increasing or decreasing the distance from the cue. An example of taxon navigation would be orienting to a flashing neon light at a pub and heading straight toward the light. Another example would be parking a car in a lot next to a light pole so that upon returning, you simply head toward the light pole. O'Keefe and Nadel stress that a taxon strategy can be highly ordered or devoid of any order at all.

Locale

The locale strategy relies on a notion of place or the place hypothesis. The locale system's representation is a group of ordered and interconnected positions within an environment. According to the place hypothesis, an animal's notion of place is obtained by activating a representation of a series of stimuli or array of cues. Accordingly, this

Table 1.1. Properties of the taxon and locale systems of navigation. (O'Keefe and Nadel 1978),p.l00.

	Taxon	Locale
Motivation for learning	Biological need: to obtain reward or avoid punishment	Cognitive curiosity: to construct and update a map of the environment
Learning change	Incremental or decremental	All-or-none
Persistence	High (esp. orientation hypothesis)	Low
Temporal changes after activation	Marked changes in threshold and strength as a function of time after activation: sensitive to inter-trial interval	Minimal changes with time after activation; insensitive to inter-trial interval
Interference between similar items	High	Low

creates a spatial representation that can be activated or used in the future to provide information.

The creation of a spatial representation is activated in two ways. Firstly, externally from two or more cues that coincide spatially. Secondly, place representations can be activated internally. The internal activation is created by another representation of place that is taken in concert with egocentric cues directing the orientation or magnitude of motor movement. Again, an example using the aforementioned pub scenario would be seeing the grocery store and Laundromat by the pub and knowing exactly where the pub is in relation to these two cues. Therefore, a sense of place is what drives animals' behaviours to explore and create a larger group of interconnected points.

iv. Cues and Stategies

Exploring animals are able to use taxon or locale strategies. When confronted with an environment consisting of mostly proximal cues, animals tend to use a taxon strategy to navigate. When confronted with an environment consisting of mostly distal cues animals tend to use a locale strategy. Because most environments consist of a mixture of proximal and distal cues, it is likely that animals use taxon and locale strategies concurrently.

v. Cognitive Maps and the Hippocampus

Disrupting the locale system by lesioning the hippocampus is proposed to result in two dysfunctions. First, the animal loses the ability to effectively explore the environment. Exploration requires a sense of place and place is dependent upon the

hippocampus. Second, a hippocampal lesion would disrupt place learning because cognitive mapping is an all or none process. Without the ability to first represent place, further place learning cannot be updated in the map. The type of information that is represented by the locale system is flexible, quick to change and easily retrieved when there is context specific information available. A taxon approach, or what is left online after hippocampal disruption is not capable of such flexibility. Deficits seen in navigation after a hippocampal lesion can be attributed to the lack of flexibility in the locale system.

Exploring animals need a large degree of flexibility and so likely are dependent on the locale system. O'Keefe and Nadel (1978) assert that ".. .in the absence of a hippocampus, all forms of organized exploration should disappear." p.242. Again, the disappearance of organized behaviour is due to a breakdown in the locale system and the rather inflexible nature of the taxon system.

vi. Subsequent Evidence for Hippocampal Spatial Involvement

Place Cells Revisited

Place Cells and Cues

The decision the brain makes about the real environment becomes more complex when space is viewed as a construct of experience. A major question that arises in studying space and navigational strategies is "how does the brain represent the physical world?" The answer to this question came from the study of hippocampal place field activity (Best, White, & Minai, 2001). After the aforementioned study by O'Keefe and Dostrovsky (1971), Ranck (1973) correlated hippocampal cell activity with various

behaviours in the freely moving rat. Two types of cells were found to have differing and unique types of activity. Firstly, pyramidal cells were noted to have complex spike patterns and secondly, interneurons were shown to fire in the 4-7 Hertz range in time with the 4-7 Hertz gradated potentials termed theta rhythms (Fox & Ranck, 1975).

O'Keefe (1976) subsequently reported finding 26 hippocampal complex spike cells that exhibited activity that appeared to be correlated with the animals' spatial location. Along with the place cells that he had found, O'Keefe (1976) also reported finding similar theta cells that fired in relation to specific behaviors as Ranck had mentioned. Following up on this research, O'Keefe and Conway (1978) manipulated various elements of the environment and found that place cells change in accordance to the specific changes in the cues. O'Keefe and Conway (1978) found that rotating cues in the environment caused a rotation in the place cells being recorded. Also, if any two cues were removed, place cells continued to fire and the fields remained constant. If three cues were removed, hippocampal place cells and their corresponding fields began to disperse and become disorganized. All of these results stimulated discussion in laboratories and spawned new research. The commonality among all the early place research was a provision of further evidence showing that the hippocampus played a crucial role in spatial processing. Thus place cells and fields are modulated at least partially by the environment.

An experiment by Shapiro, Tanila, & Eichenbaum (1997) examined the roles of different cues on place cells. They used a radial-arm maze and an environment that contained both distal and local visual cues along with olfactory and tactile cues. Animals were placed in the environment that contained a specific arrangement of these cues.

Upon achieving a standard response from hippocampal place cells, the configuration of cues was changed. The distal visual cues and all of the local cues (visual, olfactory, and tactile) were rotated by 90°. The results showed that place fields rapidly fell apart and then underwent a remapping. Further to this, 28 % of the new place fields remapped to the new location of the distal cue where as 15 % of the remapped place fields corresponded with the local cue set.

Place Cells and "Missing Cues"

From many experiments dealing with place cells and their fields it becomes apparent that distal visual stimuli play a large role in controlling the field. If the environment does play a large role in place field formation and stability then removing a cue should also affect place field stability. Therefore, experiments where cues are removed were also examined early in the place field literature. O'Keefe and Conway (1978) recorded place fields in a "T-maze" that was surrounded by curtains on all sides to control various environmental variables. Cards with visual stimuli and electronic devices that made various noises were placed in reference to various arms of the maze. When any single cue was removed the place fields remained stable. Only when numerous cues were removed at the same time did place field activity start to fall apart and become ambiguous. Therefore, these early studies and their examination of environmental cues showed that a constellation of cues were responsible for place fields and their stability (O'Keefe, 1976; O'Keefe & Dostrovsky, 1971).

Muller & Kubie (1987) also ran experiments where some cues were removed and various other environmental cues were changed. Unlike the previous cue removal experiment of O'Keefe and Conway (1978), Muller & Kubie (1987) tested animals in a

completely circular environment with a single card mounted on the wall. The cue was large enough that it covered approximately 100° of visual arc. When the card was removed, the place fields remained intact but rotated randomly. Although these results differed from O'Keefe and Conway (1978), Muller & Kubie (1987) speculated that the shape of the environment acted as a cue itself. The ability of animals to calculate their position from the edge of the table and outer walls of the room was seen as sufficient to support the place fields, but insufficient to hold the place field in a particular compass heading. All of the previous place cell testing apparatus had used curtains that ran from the floor to the ceiling and would therefore not contain this information for calculating place.

A recent cue removal experiment that varied only slightly from the previous adds further information about this process. Hetherington & Shapiro (1997) trained rats to move about in the recording chamber in search of a reward. Rather than food or water rewards that had been previously used, Hetherington & Shapiro (1997) used an electrode implanted in the lateral hypothalamus to deliver stimulation. The stimulation acted as a reward while place fields were determined. Upon achieving stable place field recordings, Hetherington & Shapiro (1997) removed various cards or changed their locations. Again, they also found that no single cue removal disrupted the place fields. Small changes in the size of the place cells were detected using more sensitive equipment when compared to that used in previous studies. These changes in the firing rates of the place cells and the resultant effects on the place fields were in relation to distance to the cue that was removed. The firing rate of place cells and the overall place fields increased if the cue being removed was greater than 30 cm away from the center of the place field.

Similarly, if a cue less than 30 cm away from the centre of the place field was removed, the firing rate decreased. The results were interpreted to mean that the hippocampus optimizes the spatial information available (Hetherington & Shapiro, 1997). This hippocampal optimization acts to increase the importance of proximal cues when distal cues are removed and decreases the effect of all cues when proximal cues are removed.

Place Cells and Darkness

Although the influence of environmental cues on place cell firing was known from these early experiments, their role in the cognitive map was not certain. The strong connection of these cells to environmental stimuli could possibly mean that place cells are simple sensory correlates of environmental cues. The further implication of this would mean that place cells and their fields did not create a comprehensive map of the environment and did not contribute to the cognitive map.

An empirical test of what place cells encoded was devised by testing animals in the dark (O'Keefe, 1976). Animals were forced to use internal representations of their space to navigate by removing all visual cues when the lights were turned off. The lack of visual cue influence on the hippocampal place fields would then predict that if place cells were environmental correlates then the corresponding place fields should disappear. If however, place cells and place fields were the major constituent of the cognitive map, then testing animals in total darkness would have no immediate effect on the place cells and fields.

The experiment was much like getting up in the middle of the night to go to the bathroom in the dark. It is easy in our familiar homes to know where the doors, stairs and other obstacles are and then to navigate effectively. The longer we walk around in the

dark, and the more complex routes we take, the more likely we are to experience a mapping error and stub our toe. The anecdotal evidence of O'Keefe (1976) showed that place fields did persist in the absence of visual cues but were found to drift or detoriate after time.

Hill & Best (1981) asked the same question about the nature of place cells encoded but used blindfolds instead of total darkness. They found that place cells did not disappear when visible cues were not available. One of the interesting results to come from this study was that place cells seemed to be formed in the absence of visual cues. To understand this finding, Hill & Best (1981) rotated the testing apparatus (radial arm maze) and found that the place cells rotated with the maze. The rotating of the place cells suggested that olfactory cues were salient enough to not only maintain place fields but create them. Hill & Best (1981) concluded from these results that rats can use other salient cues when distal cues are not available. A result that was not readily explained from this experiment was that a few of the place fields remained intact and did not rotate with the rotation of the radial arm maze and its olfactory cues. These intact place fields suggested that another form of information was still available for animals to update and maintain their place fields with.

Quirk, Muller, & Kubie (1990) attempted to answer the question about these remaining place fields by filming rats in total darkness using infrared video and flashing light emitting diodes (l.e.d.). In this experiment rats were given pre-training where the lights were turned off halfway through each training session. Once rats had become accustomed to the task, and place fields were stabilized, animals were placed in various groups that differed in their exposure to the environment. A typical testing session

consisted of animals foraging for food pellets in one environment for 8 minutes and then the lights were turned off for 8 minutes. During the final 8 minutes of this exposure to this first environment animals had the lights turn on again. This exposure was called the light-dark-light session (LDL). The LDL exposure was then re-administered for a second testing cylinder. Animals were then placed back in the first apparatus for eight minutes in the dark followed by second 8 minutes in the light (DL). A second DL session was then repeated in the second testing cylinder. All animals received a trial that consisted of a LDL, LDL, DL, and DL where the testing cylinder was changed between each session.

The place fields were then examined across all of the testing sessions. The L segments of the different sessions had place fields that were the most similair. Most place fields also remained constant between the shift from light to dark and vice versa. Place fields were found to deteriorate rapidly between the second LDL session and the first DL session. When the place fields deteriorated after the first dark phase of the first DL session, the re-mapping was then found to persist in the light phase of that session.

These results suggested that although place fields showed disruption and re-mapping between the dark and light sessions of different environments, these re-mappings continued into the next phase. This result further suggested that place fields appeared to be contingent mostly upon recent experience more than anything else (Quirk, Muller, &Kubie, 1990).

Food-Storing in Birds

Many animals that live in seasonal climates store food. Considering all the complexities that are involved in navigating in an environment, it is amazing that birds

accurately remember and retrieve their stored food. The number of food items that can be stored ranges from ten thousand to more than two hundred thousand (Vander Wall, 1990). A greater significance than the number of food caches used is the fact that most are remembered. For periods as long as 7 — 11 months, nutcrackers have been shown to be quite capable of remembering cache locations (Tomback, 1980).

A close examination of the movement components made by nutcrackers as they cached and retrieved food found that many components were similar (Kamil, Balda, & Good, 1999). Disrupting movements during the retrieval phase by placing objects in the path taken during caching significantly decreased the number of cached items retrieved (Basil, 1993). Basil (1993) has shown that when nutcrackers where trained on a set of 9 distal cues, animals were successful in retrieving cached items when three of the nine cues were presented.

In the early 1960's, a Russian ethologist discovered that food storing birds that had lesions to the medial telencephalon lost the ability to retrieve food that had been cached (Krushinskaya, 1966). Following this research, Krebs, Sherry, Healy, Perry, & Vaccarino (1989) discovered that hippocampal lesions in chickadees, a food storing bird, also disrupted the retrieval of cached items. Interestingly, chickadees with lesion still cached food but could only retrieve a few items and only when they were in very general locations.

Sherry & Duff (1996) state the key item remembered by birds is the relationship between landmarks and the places where food is cached. Salient and unchanging cues in the environment served to direct caching behavior. Brodbeck & Shettleworth (1995) and Sherry & Duff (1996) propose that cues represent "global, local to featural information...

[and] permit efficient searching at successive finer scales of resolution" p. 170. These results suggested that the landmark features of an environment (locale) were more important than the finer (taxon) characteristics of the environment.

Another avenue of research on food storing in birds is the relationship between this behaviour and the volume of the hippocampus. The ability to remember large quantities of spatial information means that anatomical areas that are correlated with spatial behavior need to be larger. Barnea & Nottenbohm (1994) found that during autumn, when food storing takes place, hippocampal volume was the greatest in chickadees. The evolution of such a trait allows for a greater number of food items to be stored. The increased demand for spatial abilities was seen to be related to the increase in hippocampal volume, seems to be a special adaptation in small animals with constrained brain size.

Human Imaging

Previous research has shown that in addition to birds, various species of animals have seasonal enlargement of the hippocampus when there is an increased spatial demand (Smulders, Sasson, & DeVoogd, 1995). Human functional imaging studies show similar results to the bird food storing studies. Maguire, Frackowiak, & Frith (1997) examined topographic memory retrieval in taxi drivers to see if particular brain regions were highly active and therefore involved. They were interested in how subjects used landmarks and the role of configurations of landmarks in navigation. The study set out to clear discrepancies between research stating the posterior parietal cortex was key for landmark orientation (DeRenzi, 1977) and research stating the medial temporal lobe is the spatial

processing center (Maguire, Burke, Phillips, & Staunton, 1996). The researchers used landmarks that were well known but that had little spatial context or a spatial context that was unknown.

Maguire, Frackowiak, & Frith (1997) examined positron emission tomography images from London taxi drivers. The significance of using London taxi drivers is that they must train for three years and pass a test before becoming a certified and licensed driver. Having all been tested in London, all taxi drivers were familiar with the same important landmarks. Maguire choose 11 right-handed males who were around the age of 45. The average time that each male had spent as a taxi driver was between 14 and 15 years.

Subjects were given six tasks and performed each of the tasks twice. Out of the six tasks, two tasks involved recalling information about landmarks in the London area. In the first of two landmark tasks, drivers were asked to find the shortest route to a goal destination. The route could not break any traffic laws and started from a specific location. The second part of the landmark task was to describe major features and tourist destinations along the route (Maguire et al., 1996). The first part of the landmark task involved recalling stored information in a specific sequence where as in the second part, the amount of details stored was of much greater importance. The other four tasks involved non-spatial forms of memory and included tasks such as recalling the plots from films that subjects were shown. Subjects were then screened to see the various areas of London that they were most familiar with and the films that they had seen most recently. During the administered tasks, all subjects were scanned with a positron emission tomography (PET). All PET data analyzed was smoothed using an isotropic Gaussian fit.

The fit allowed for corrections in cerebral blood flow to be accounted for and further error to be eliminated from the imaging process.

Maguire et al., (1997) report finding a main effect of increased right hippocampal activation during route navigation. When route navigation and other tasks that involved landmark use were compared, an increase in cortical activation was seen by the route navigation tasks. In particular, the medial parietal cortex seemed to be involved, where as the posterior cingulate was not as active in the landmark task as in the route finding task.

A second finding from the study was the apparent difference between the navigation tasks and the recall from specific films. There were increased regions of activation in the subjects during the navigational task as compared to the non-navigational tasks. The medial parietal, posterior cingulate cortices, fusiform gyri, and the parahippocampal gyri were all comparatively more active during the navigation tasks.

The findings of Maguire et al., (1997) are complimentary to earlier recording because they link hippocampal function to the mapping of space. The hippocampus had previously been found to not be involved in computer simulated environments (Aguirre, Detre, Alsop, & D'Esposito, 1996). Using a real world environment creates a greater degree of complexity that accounts for the recruitment of the hippocampus in navigational tasks (Maguire, Frackowiak, & Frith, 1997; Thompson & Best, 1989). The overall results of these studies are that the right hippocampus is recruited for navigational tasks and that other areas, the medial parietal cortex, posterior cingulate cortex and the parahippocampal gyrus are all involved in complex navigational decisions.

Hippocampal Dependent Tasks

Radial-arm maze

One of the first maze tasks that attempted to test memory of locations was the radial-arm maze developed by Olton & Samuelson (1976). The original version consisted of eight-arms projecting out radially from a central location (Fig. 1.4) and was thus similar to Tolman's sunburst maze. During a typical trial each arm of the maze contained a food reward. Rats were placed in the central arena of the maze and allowed to freely enter all of the arms until the food was consumed. Because animals try to obtain all the food without wasting any effort, they soon learn that the optimal strategy for obtaining all the food with the least amount of effort. The least amount of effort consists of visiting each arm only once during a trial. When examining the behaviour and memory capabilities, arms that are re-entered after rats have already consumed the food are scored as errors because they are a waste of time and energy. From this experiment and others similar to it, Olton concluded that animals use a type of spatial-working memory (Olton & Papas, 1979). This type of working memory was defined as a short-term storage that enabled rats to keep track of which arms they visited on a given trial. This type of memory was labeled short term because it was only needed for the duration of the trial, and therefore a short time.

Additionally, retrograde and anterograde working memory consolidation can be tested by using a slightly modified apparatus. The original maze design can be altered by placing partitions in front of each arm. After animals have visited a few of the arms and received the food reward, the partitions are lowered and the animals are subjected to a

Figure 1.4. Photograph of the radial arm maze. The radial arm maze is composed of eight arms extending out from a central circular arena. Animals are typically allowed to freely explore the maze, consuming food rewards located at the end of each arm. This type of maze is generally used to test memory function.

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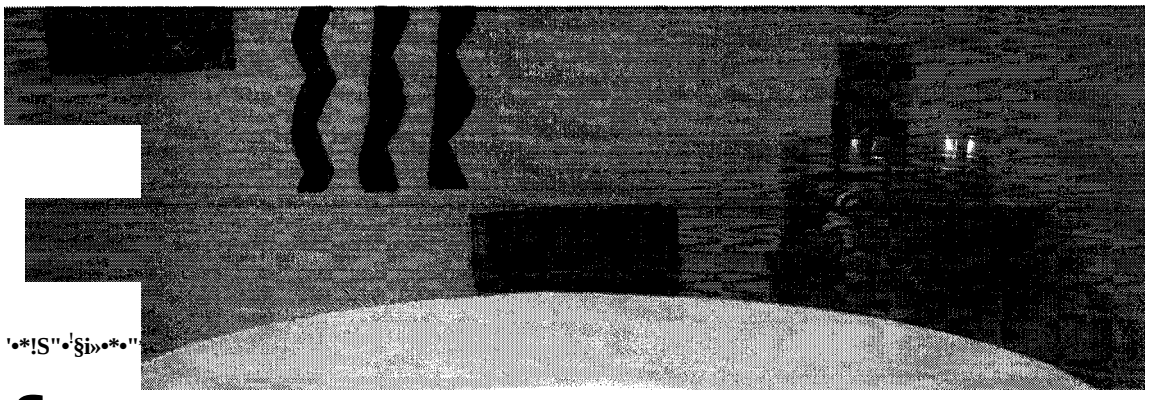
time delay until their next trial and consequent arm choice. Retrograde working memory errors are made when arms entered prior to the time delay are entered again, while antrograde working memory errors are made when arms entered after the time delay are re-entered (Olton, 1983). Regardless of the actual radial arm apparatus, rats with lesions to the hippocampal system show an inability to obtain all of the food rewards in the radial-arm maze without numerous repetitive errors (Olton, 1983; Olton & Papas, 1979).

Swimming Pool Task

The swimming pool task (water maze, Morris water maze) was developed as a an alternate to the radial arm maze of Olton (Morris, 1981). The water maze offered new insight in that it was cable of answering questions about spatial working memory that were not readily teased out by the radial arm maze. Again, like the radial arm maze, there have been many forms and adaptations of the swimming pool task.

In the water maze's basic form, animals are released from one of four cardinal compass points around a circular pool (Fig. 1.5). The pool is filled with water, and powdered milk or tempera paint is added to make the water opaque. Animals are expected to find a platform submerged just beneath the surface. The submerged platform is not visible and after numerous trials animals learn the location of the hidden platform based on distal cues. As animals learn the task, their escape time or the latency to escape decrease greatly. Animals are then given a probe trial where the hidden platform is removed and the amount of time spent in the old platform quadrant is quantified. Large amounts of time spent in the vicinity of the old quadrant represent learning of that location as the escape from the maze (R. G. M. Morris, 1981; Sutherland & Dyck, 1982).

Figure 1.5. Photograph of the swimming pool task. In the swimming pool task, animals are released from one of the four cardinal compass points into a circular pool. The pool is filled with water, which is made opaque by adding powdered milk or tempera paint. Animals are expected to find a hidden platform submerged just beneath the surface to escape the water.



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The water-maze is also used with a visible platform above the surface of the water as a control to ensure that animals do not have motor or visual problem. This ensures that animals are in fact capable of swimming to the platform. This visible platform water maze does not require the rats to remember the location of the platform and therefore any impairments are not related to spatial memory. The Morris water-maze has an advantage over the radial maze because it is able to demonstrate the ability of rodents to locate a place without direct guiding cues from visual, auditory or olfactory cues.

The acquisition of place in the water maze has been found to be sensitive to lesions of various anatomical structures. Hippocampal lesions (Sutherland, Kolb, & Whishaw, 1982) and selective lesions of the dentate gyrus (Sutherland, Whishaw, & Kolb, 1983) have been shown to disrupt the initial acquisition. Acquisition is also disrupted by lesions downstream of the hippocampus, in the subicular complex (Morris, Schenk, Tweedie, & Jarrard, 1990) and medial frontal cortical region (Sutherland et al., 1982). Lesions upstream to the trisynaptic circuit, and specifically the perforant path, disrupt the entorhinal input and also disrupt acquisition of the place response in the water maze (Skelton & McNamara, 1992). The medial septum is another major input to the hippocampus and has also been shown to disrupt acquisition of place in the water maze (Hagan, Salamone, Simpson, Iversen, & Morris, 1988).

Some of the behaviours exhibited in the water maze and the direct role of the hippocampus in the task are still unclear, however Whishaw, Cassel, & Jarrad (1995) have shown that when rats with fimbria fornix lesions are given extensive visible platform training, they show spatial learning by heading for the hidden platform. In the same experiment, when the platform was switched to a new location, the fimbria fornix

lesioned animals are not capable of learning the new position. The Whishaw et al., (1995) experiment suggests that there is a further distinction between learning the appropriate strategy for getting there and learning a strategy of knowing where. Whishaw et al., (1995) conclude that animals with hippocampal lesions are capable of learning where the platform is even though they display impairments in getting there.

Delayed non-matching to sample

Although the major concern of this thesis will be with rodents, and specifically rats, a large portion of the hippocampal learning literature is based on primate lesion studies. Delayed non-matching to sample (DNMS) is one of the tasks on which primate subjects with a lesioned hippocampus show consistent impairments (Mishkin, 1978). The first trial of the DNMS task starts with two objects being presented to the subject. When one of the objects is chosen the subject receives a reward. The second trial then pairs a novel object with the previously rewarded object. In the second trial the reward is given for the novel item. Therefore, the task requires a subject to make a decision based on which object appears novel. A number of studies have found that this process appears to have exclusive hippocampal involvement (Mishkin, 1978; Zola-Morgan & Squire, 1985).

///. *Ethological Space*

i. Exploration

Although many publications and papers have been concerned with the spatial abilities of various animals, a theory of exploration that examines all phenomena is

lacking. Shillito (1963) studied the behaviour of voles (Microtus agrestis) and found that voles reacted to objects that were unfamiliar to them. Shillito went to great extremes to design her laboratory so that the living conditions of voles would mimic that of their natural habitat. Observations of the voles were made while they were living in their common areas and also in novel areas to see the role the environment played in their exploratory behaviour.

Exploratory behaviour was defined as "that behaviour which serves to acquaint the animal with the topography of the surroundings" (p. 146). The repertoire of exploratory behaviours all seems to highlight a need for maximum sensory exposure. This maximum sensory exposure allows the animal to use all of it's sense to acquire large amounts of detail about the environment quickly. Once an animal has explored excessively, the exploratory bout stops.

When first placed in a novel environment the first observable behaviour was to "huddle" or hide in a corner of the environment. Shillito (1963) describes this first part of exploration as "more or less random" (p. 147). To determine where animals spent most of their time, paper was placed on the ground to track movements. It was noted that once a particular object was visited, animals made less frequent trips back to that object. The ability to recognize new objects and the degree of familiarity in an environment can be seen in the importance of behaviours that guide new object recognition. Knowing what is new in an environment can be seen as an adaptive advantage because it allows animals to be aware of their topography and therefore avoid predators and conspecifics along with finding food and mates (Shillito, 1963).

Shillito further states that there are two typical situations in which exploration is employed. In the first situation, voles explore because they are in a new situation or environment and this situation is consequently driven by external stimuli. The second type of exploration is driven by internal stimuli. According to Shillito, the difference between the two types of exploration is determined by the speed in which they execute the exploration. Novel stimuli require more processing for the vole and therefore the speed that they explore decreases. The overall organization of exploration appears to still be quite random in the Shillito's results.

ii. Exploration and the Home Base

The same behaviour that was thought to lack organization by Shillito (1963) was seen to be very organized by Eilam & Golani (1989). The behaviour that seems to appear the most frequently during exploration is the creation of a home site or home base. When trying to understand how exploratory behaviour was organized, Golani(1989) felt that animals use a series of reference points to guide their spatial behaviours. The high proclivity for animals to create a home base, coupled with the necessity for shelter and other biological needs, make the home base a reference point. Again, the emphasis of Golani's research was to describe the occurrence natural behaviours. The researchers acquired first generation wild rats and handled the animals so that anomalies due to anxiety and fear were lessened upon placing the animals in the testing apparatus.

The testing apparatus was a 160 x 160 cm glass platform. The transparency of the glass allowed for a camera to capture the movements of the rats from below. Movements like crouching and rearing were readily quantified in this manner. A camera behind a

hide also helped to record the general paths of the rats. All of the exploratory bouts lasted one hour.

Animals displayed an organized pattern of progressions and stops immediately upon being placed in the novel environment. The rats were found to prefer one or two places and the stops of greatest duration were spent in one of these two locations. The place where rats spent most of their time was defined as the home base and was typical durations were 10 times larger than any other place at this location (p. 208).

A second indicator of the home base was that typical behaviours were displayed in this region. Grooming and rearing were two behaviours that had higher incidences at the home base. Animals were seen to exhibit these two behaviours to a much greater degree only at the home base area. The animals also spent most of their time at the home base, however this finding might reflect the amount of time spent there. In order to avoid creating a bias related to the length of the exploratory session, Eilam & Golani, (1989) examined the behaviours in relation to the proportion of time. The proportion of time spent grooming at the home base was higher than any other location even when amount of time spent at the home base in total was factored in. When rearing at the home base was examined in relation to the amount of time spent at the home base, no difference was found. This means that although rearing and time spent at the home base have a somewhat linear relationship, the relationship between grooming and time spent at the home base is not as simple.

Another indication that a location may serve as a home base is the number of visits or trips that occur to that point. It was found that although rats visited a number of positions in the novel environment, on average rats make more visits to the home base

location. The number of visits is a measure of frequency that is not dependent on time and so also serves as an independent measure. Because the home base appears central, the objectives of the present experiments is to further examine how rats locate a home base.

IV. Objectives of the Present Study

The main objectives of this thesis were to determine (1) if proximal cues are involved in the formation of a home base and (2) if distal cues also play a role in home base formation. A determination of (3) self-movement and olfactory cues in home base formation was also examined and acted as a control to see if the home base was formed exclusively by visual cues. The role of the home base in spatial learning was also examined using both Proximal Cue Probes (4) and Distal Cue Probes (5). In concert with all the aforementioned objectives, the role of the hippocampus was examined using a group of hippocampectomized rats to examine it's role in these specific spatial behaviours.

The objective of the first experiment was to investigate whether proximal cues have a role in the formation of a home base. In conjunction with this, a group of animals with hippocampal lesion also investigates the necessity of the hippocampus in the formation of a home base. Animals were placed a large circular table and allowed to freely explore. The environment contained a large cue that was proximal the table. Measures of time spent in various areas, speed and distance were all calculated for each rat. These measures and others are described in detail in the following chapters

The objective of the second experiment was to investigate whether distal cues have a role in the formation of a home base. Control rats and rats with hippocampal

lesions were tested in the same room, but distal cues were manipulated. The most prominent distal cue was a bookcase, and so for all trials this cue was present, removed, or enhanced. Similar measures to the first experiment were calculated for each rat and are described in detail in chapter 4.

The objective of the third experiment was to investigate whether self-movement and olfactory cues have a role in the formation of a home base. In conjunction with this, a group of animals with hippocampal lesions also serves to investigate the necessity of the hippocampus in home base formation in total darkness. Control and hippocampectomized groups are compared to animals with olfactory bulb lesions for their ability to form a home base in total darkness. In addition to the aforementioned measures, a stop dispersion index was used to determine how tightly grouped the animals stops were. The details of the third experiment are elaborated upon in chapter 5.

The objective of the fourth experiment was to examine whether the home base behaviour involves learning. Rats were trained to a home base location over a series of four days using a proximal cue. On the fifth day of training, the proximal cue was removed and animals were tested to see if they had learned the location of the home base be relating the amount of time spent in the quadrant that had previously contained the cue. The details of the fourth experiment are elaborated upon in chapter 6.

The objective of the fifth experiment was to examine whether the hippocampus is necessary for home base behaviour when a distal cue is manipulated. Rats were first exposed to an environment with a salient distal cue for four days, and then the cue was removed and animals were tested on the fifth day. The fifth experiment examined whether rats could form and maintain a home base in response to distal cues after a

salient distal cue is removed. The details of the fifth experiment are elaborated upon in chapter 7.

A final summary of all the results and the further implications of all five experiments is discussed in the final chapter, chapter 8. The role of cues and ofthe hippocampus in home base behaviour is discussed and future directions are mentioned.

CHAPTER 2
GENERAL METHODS

/. *Animals*

The subjects in all experiments were female Long-Evans rats (University of Lethbridge vivarium) and each weighed from 200-300 gm. All rats were housed with at least one other rat in plexi-glass cages. The room in which animals were housed had a constant room temperature between 20-21°C with a 12 hr light/dark cycle. Rats were fed with Lab Diet Laboratory Rodent Pellets in their home cage and allowed free access to both food and water.

77. *Surgery*

Rats were anesthetized with a mixture of isofluorane and oxygen (4% with 21 per minute of oxygen and 2% after a surgical plane was established.) An incision was made in the scalp and the periosteum to expose the cranium. A dental burr was used to drill small holes in the skull at specific coordinates. Coordinates were obtained by placing animals in a stereotaxic device and tarring all measurements from a fixed position, bregma, on the skull. Coordinates for the surgeries were modified from (Sutherland et al., 2001) to accommodate for use with female rats. The injection sites were as follows: 3.1, 4.0, 5.0, 5.3, 6.0 mm posterior to bregma, 2.0, 3.0, 3.5, 5.0, 5.2 mm lateral from bregma, and 3.6, 3.5, 3.5, 7.3, 5.5, 7.5mm ventral to the surface of the brain. All cooridinates are in the sequence that they were performed and injections were made on both sides of the brain to make a bilateral hippocampectomy. The hippocampus was

selectively damaged using a N-methyl-D-aspartate solution (10 mg/ ml). Rats were administered 0.4 *il* at each of the 12 injections sites. Animals were given diazepam (15 mg/kg i.p.) as soon as muscle torpor was demonstrated to counteract the seizure activity created by the procedure. Animals also received the opiate bupenorhpine (10 mg/kg i.p.) to alleviate pain. Control animals did not undergo any surgical procedure. Animals were given two weeks to recover after the surgery before behavioural testing began.

///. *Apparatus*

Foraging table: the apparatus was a wooden circular table without walls measuring 244 cm in diameter (Fig. 2.1). The table was painted white and contained a motor mounted on ball bearings that permitted the table to be rotated in both directions and at varying speeds. The surface of the table was approximately 64 cm above the floor. The table was cleaned with both an antiseptic solution and a dilute ammonia solution to remove any contamination odor cues left by preceding rats. All behaviours were video recorded from above using a Sony HI-8 camera that was mounted perpendicular to the table.

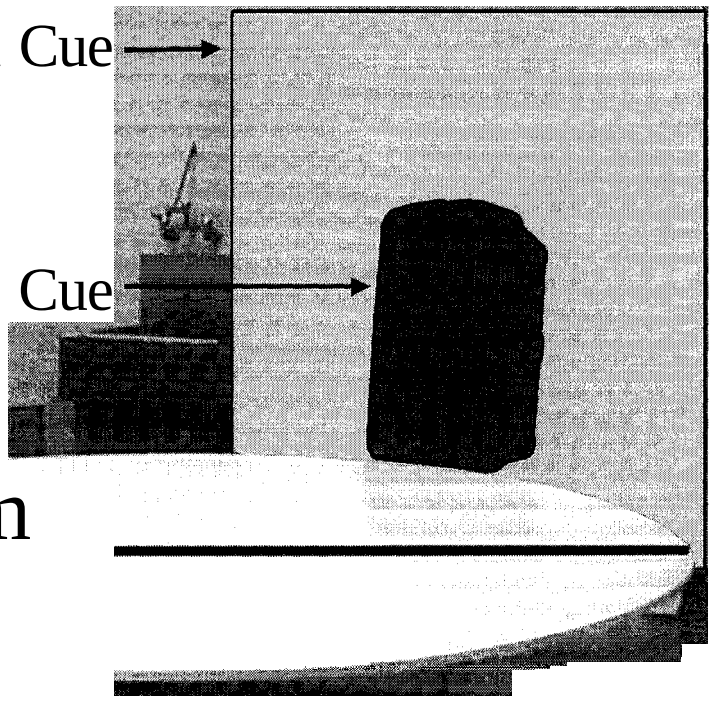
i. Infrared Testing

The table was located in a large room that could either be illuminated or made completely dark. The testing involving complete darkness was accomplished by using a Sony infrared camera positioned perpendicular to the table. Previous research by (Neitz & Jacobs, 1986) has shown that infrared is a wavelength of light that rodents are not

Figure 2.1. Photograph of the general apparatus used. The foraging table was a white circular table, 244 cm in diameter. In the Proximal Cue experiments, a large black box is placed adjacent to the table. In the Distal Cue experiments, the bookcase against the back wall was used as a cue.

Distal Cue

Prdximal Cue



244 cm

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capable of detecting. The testing room was light-proofed prior to testing, so that the room was completely dark. Animals that were tested in the dark were given 10 minutes of darkness prior to the trial to become accustomed to the dark. Infrared spotter goggles were used when the experimenter was in the room during the dark testing. Two infrared illuminators were also positioned in the room to increase the amount of infrared light available and therefore increasing the brightness of the recorded video and allowing for a higher degree of resolution.

ii. Digitizing

Exploratory trips that were to be analyzed later were converted from analogue recording to a digital computer file using the Accu-Trak (Columbus, Ohio) system with a sampling rate of 60 frames per second. The exploratory path traveled by the rat is acquired from the digitized file by sampling the x- and y-coordinates of the rat as it moves. The data reflects moment- to-moment movements of the animals during the exploratory bout. The spatial coordinates were calibrated using a known measurement so that all distances and speeds were actual measures.

IV. Histology

After testing, animals were deeply anesthetized using sodium pentobarbital and perfused transcardially with saline. A saline-formalin (10%) solution was then perfused transcardially to fix the tissue. Each brain was removed from the skull and stored in 30%

sucrose-formalin solution to cyro-protect the tissue. The brains were frozen and cut at 40 jim on a cryostat. Alternate sections were taken and stained with cresyl violet.

V. Data Analysis

All x-y coordinates were processed using the program language C++ (see appendix). The distance and consequent speeds and accelerations for each exploratory bout were calculated using the distance formula:

$$distance = y/\{x\backslash — x\text{-}®)^2 + (t\text{^} — fi_0)^2$$

Stops times were calculated by a simple filter that placed xy coordinates in a filter bin according to the amount of time they occupied. So, if a set of xy coordinates first satisfied the criteria for a stop, it was then next placed in a bin according to its duration. The filter bins were less than a second, 1-3 seconds, 3-10 seconds, 10 seconds to 30 seconds and greater than 30 seconds.

The dispersion between stops was calculated by drawing a geometrical figure between any three stop coordinates and factoring in the space between all of the possible geometrical representations. The formula used to calculate this dispersion was:

$$^{D} = w{=}z\ E"{=}i\ E"{=}i\ V(^{X}J\ -\ ^{x}*)^2 + (B \sim \wedge)^{2\ where\ i} \wedge 3$$

CHAPTER 3
EXPERIMENT 1 - ROLE OF PROXIMAL CUES & THE HIPPOCAMPUS

7. *Introduction*

The contrasting ideas of Shillito (1963) and Eilam & Golani (1989) raise a question regarding the exploratory behaviour of rats. From the prospective of Shillito (1963), the exploratory behaviour of rats is random. Eilam & Golani (1989), however, find the exploratory behaviour of rats to be organized around a central environmental location; the home base. Although Eilam & Golani (1989) posit the home base as an organizing mechanism for exploratory behaviour, they conclude that the home base is arbitrarily chosen by rats.

An interesting quality of the Eilam & Golani (1989) testing environment is that they went to great lengths to make it devoid of visual cues. Although they use an ethological approach, the natural world is rarely homogenous. It is quite possible that the homogenous testing field is what made an animals choice of home base location appear random. Place cell research suggests that if cues do play a part in organizing the environment then this orgaziniztion should have a basis in the hippocampus (O'Keefe & Nadel, 1978).

The present experiment was designed to investigate whether proximal cues have a role in the formation of a home base. In conjunction with this, a group of animals with hippocampal lesion also investigates the necessity of the hippocampus in the formation of a home base.

//. *Methods*

i. Proximal Cue

A total of 12 female Long-Evans rats were used assigned to either a control (n=7) or hippocampal (n=5) groups. In the Proximal Cue condition, a large black box (80 x 47 x 42 cm) was placed 30 cm back from the edge of the table (Fig. 3.1). The bottom of the box was 66 cm off the floor or roughly at the same height as the table. Animals were placed in the center of the table facing randomized compass positions. Trials were two hours in duration and subjects received one trial per day over a four-day period. The table was divided into four imaginary quadrants and the cue was moved to each of these quadrants in a randomized fashion over the four trials.

ii. Swimming Pool Task

The swimming pool was located in a test room (315 cm x 620 cm x 300 cm high) in which numerous cues were present (posters, counters, pipes; Fig. 3.2). A 156-cm-diameter and 46 cm high, round white swimming pool was positioned 47 cm above the floor and filled with 21-22°C water that was made opaque by adding 750 cm of powdered milk (Sutherland et al., 1983). A clear Plexiglass platform with an area of 11 cm^2 was placed so that the top was 1 cm below the surface of the water, where it was not visible to a viewer on the surface.

Place Task

Animals were tested two trials per day for ten consecutive days, with the platform always located at the center of the southwest quadrant of the swimming pool

Figure 3.1. Photograph of the Proximal Cue apparatus used. In Experiment 1, a large black box (80 x 47 x 42 cm) was placed beside the foraging table to act as a Proximal Cue.

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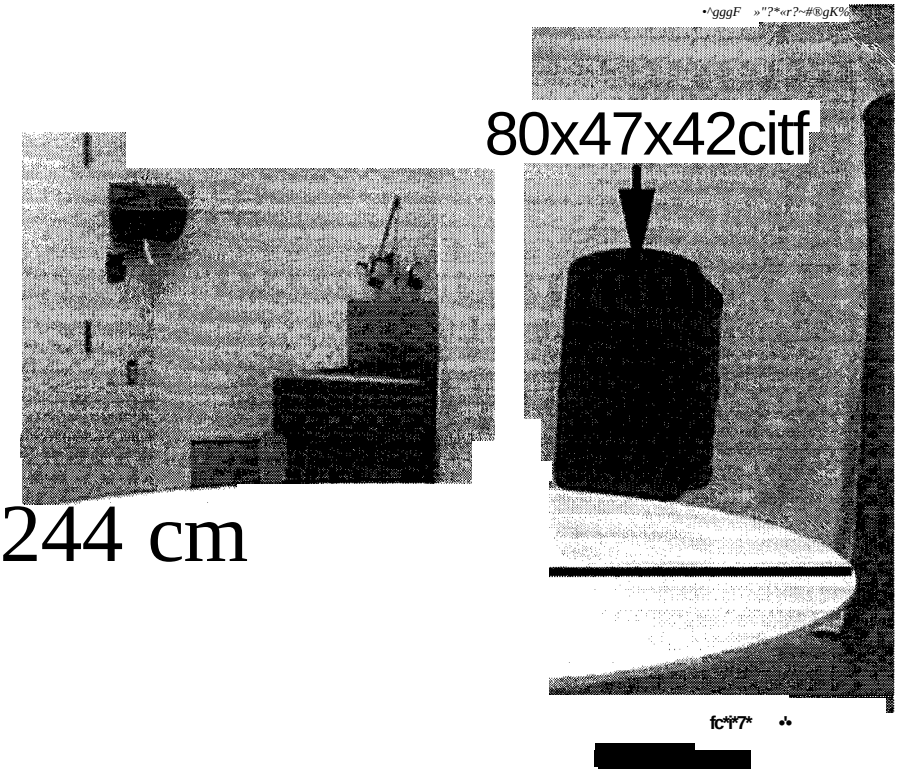

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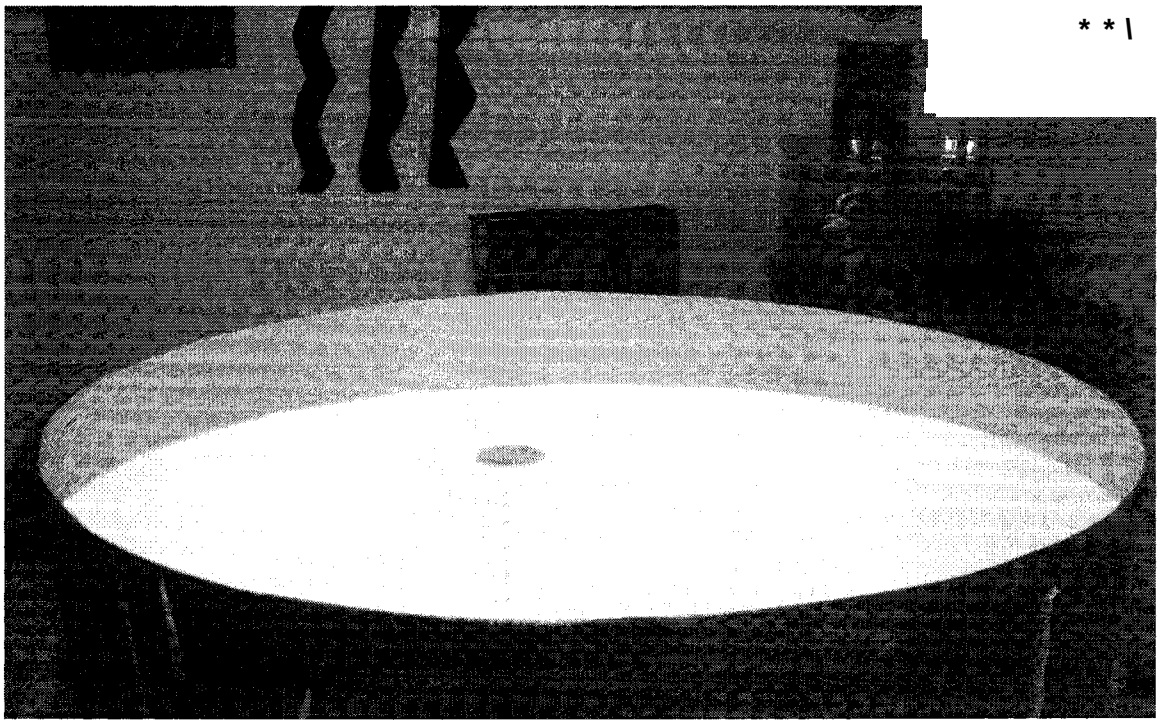
Figure 3.2. Photograph of the Swimming Pool task apparatus. Swimming pool task requires animals to spatially locate a hidden platform, relative to environmental cues.



facing the wall of the pool, at one of the four starting positions (north, south, east, and west) around the perimeter of the pool. All four start positions were distributed equally among subjects on each trial, with the order of the start position for any given subject occurring in a random manner. If on a particular trial the rat found the platform it was allowed to remain on the platform for 10 sec. If after 90 sec the rat failed to find the platform, it was then guided to the platform and permitted to remain for 10 sec. At the end of the trial rats were returned to the holding cages and 10-20 seconds elapsed before the next trial was started. Measures of swim latency were recorded.

Matching to Place

Animals were tested two trials per day for 5 consecutive days, with the platform moving to a new location each day (Whishaw, 1985). The starting position for any given subject remained the same for both trials on any given day. Again the four start positions were randomized for all subjects. Rats were placed in the swimming pool in the same manner as for the place task. During the matching to place task, however, rats were required to swim until they found the platform, where they remained for 10 sec, and were then placed in the holding cage for 20 sec before starting trial two.

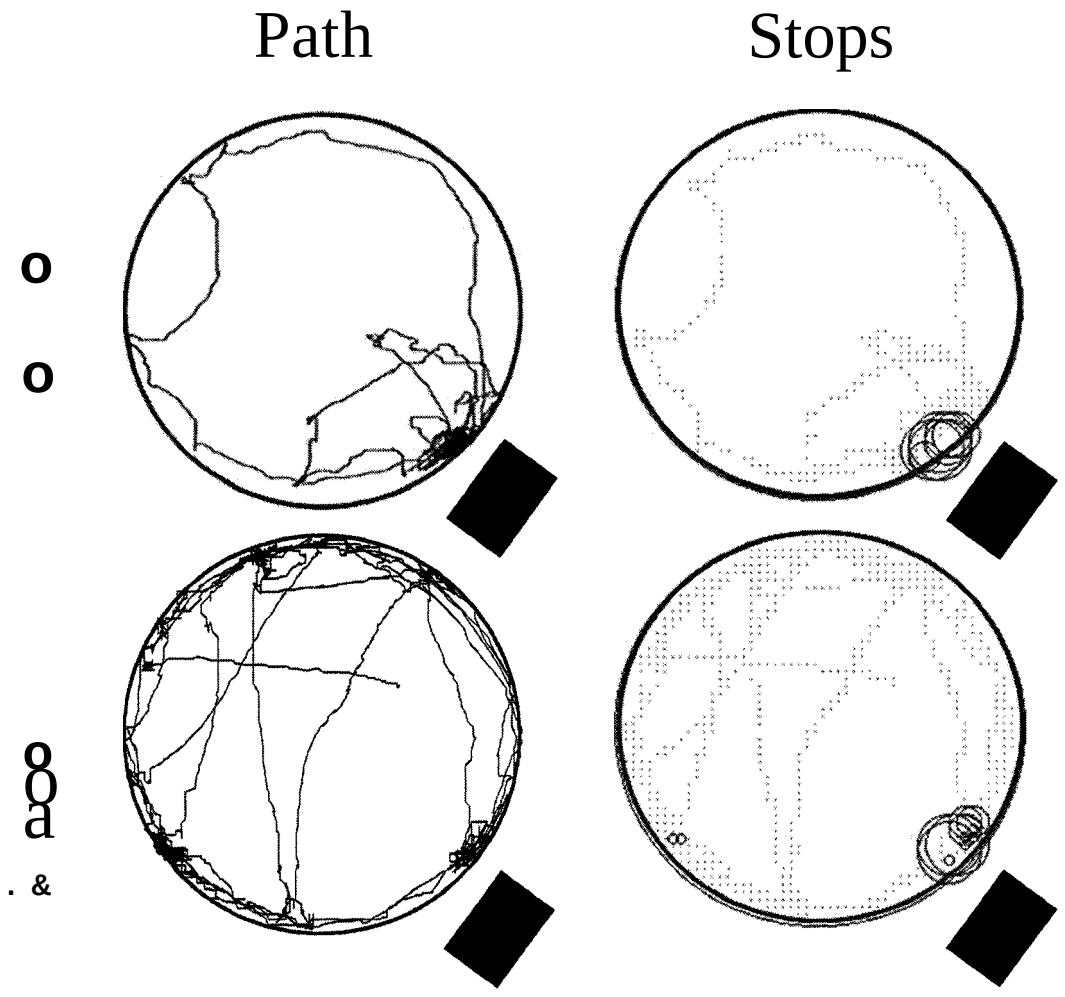
III. Results

i. Paths and Stops

Interpretation of the paths and stops revealed the location of the home base to be influenced by the cue (Fig. 3.3). Animals set up their home base in front of the cue,

Figure 3.3. Paths and stops with the Proximal Cue, for typical control and hippocampectomized rats. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. Stops are represented by circles, with diameters corresponding to the amount of time spent at that location. This pattern of behaviour suggests that animals set up their home base in front of the Proximal Cue.

Proximal Cue



represented by circles with diameters corresponding to the amount of time spent at that location. Typical home base behaviours were also seen at these locations.

Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. The direct nature of paths around the Cue further highlights that the rats create a home base in front of the Cue.

ii. Quadrant Time

A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect of quadrant ($F(3,39)=41.073$; $p<0.0001$; Fig. 3.4). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparison (Bonferroni LSD; $p<0.0001$; Table 3.1) revealed that the Cue quadrant was where animals spent the majority of their time. The quadrant effect was only seen in the Cue quadrant and animals had a mean of total time 5193.57 seconds (±403.13) per day. These results demonstrate that both control and hippocampectomized animals spend most of their time in the Cue quadrant. Coupled with an examination of the animals' paths and stops, we further see that animals are stopped in front of the Proximal Cue.

iii. Quadrant Distance

A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant quadrant effect ($F(3,39)=4.64$; $p=0.007$; Fig. 3.5). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x

Figure 3.4. Mean and standard error of the total time spent in each quadrant. This graph demonstrates a significant effect of quadrant ($F(3,39)=41.073$; $p<0.0001$).

Proximal Cue: Quadrant Time

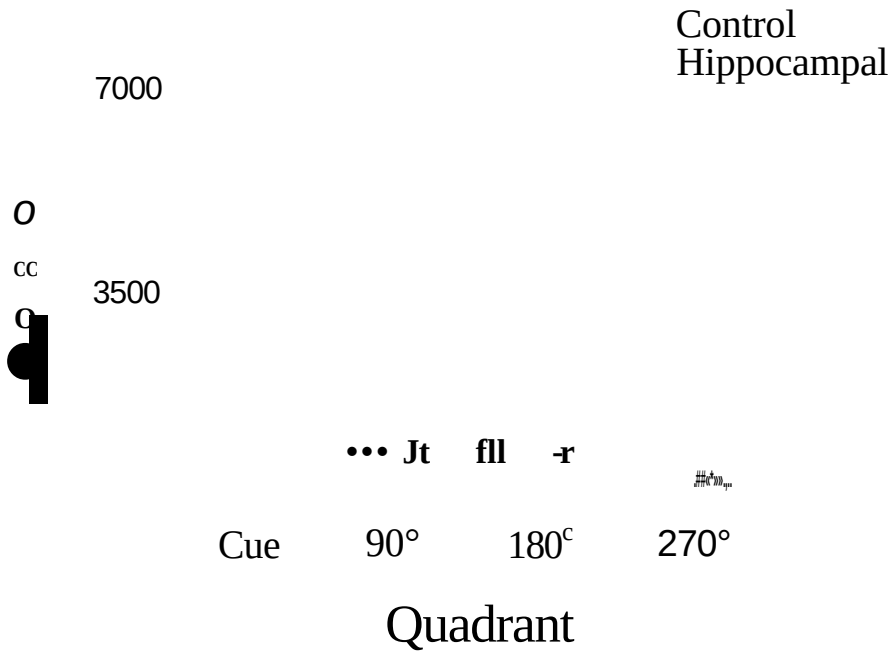
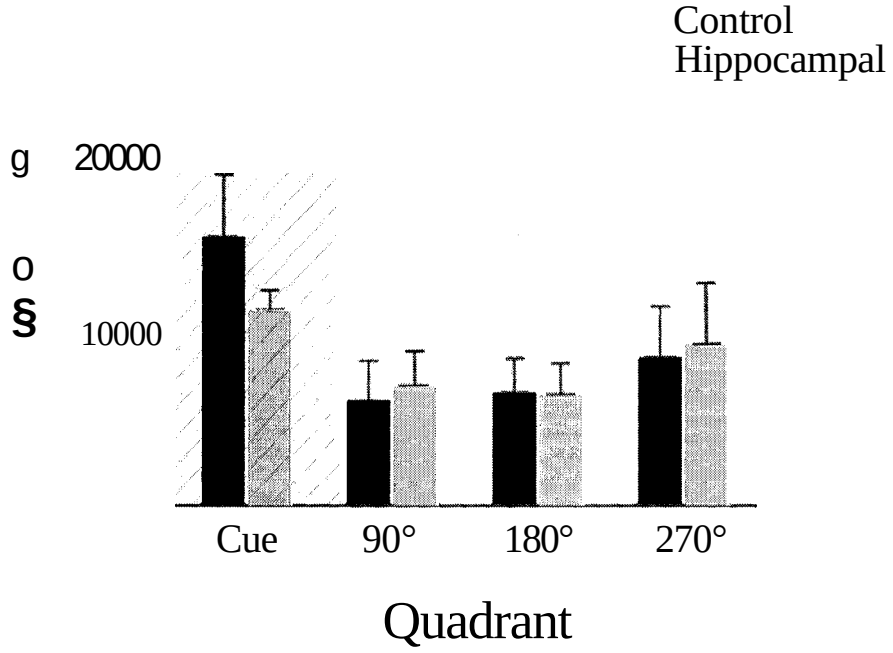


Table 3.1. Post Hoc comparison of the total time spent in each quadrant in the Proximal Cue experiment.

M H 4 If**	Cue	90°	180°	270°
Cue	** ◸ 3	***	***	***
90°	*#*			—
180°	***			—
270°	***		—	

Figure 3.5. Mean and standard error of the total distance traveled in each quadrant. This graph demonstrates a significant effect of quadrant ($F(3,39)=4.64$; $p=0.007$).

Proximal Cue: Distance



quadrant x group effect. Subsequent multiple comparison (Bonferroni LSD; $p<0.01$; Table 3.2) revealed that the Cue quadrant was where animals traveled the least distance. The quadrant effect was only seen in the Cue quadrant and only different from the quadrant 90° and 180° from the cue. Animals had a mean of total distance in the Cue quadrant of 13282.10 cm (±1773.17) per day. These results also show that both groups, control and hippocampectomized animals, traveled significant less in the Cue quadrant. Coupled with the significant amount of time that animals spent in the cue quadrant, animals are largely immobile in the Cue quadrant.

iv. Quadrant Speed

A within subjects analysis of variance on the average speed in each quadrant revealed a significant quadrant effect ($F(3,39)=12.526$; $p<0.0001$; Fig. 3.6). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparison (Bonferroni LSD; $p<0.001$; Table 3.3) revealed that the Cue quadrant was where animals traveled at the slowest speeds. Animals had a mean of speed in the Cue quadrant of 1.72 cm/second (±0.58) per day. The significantly lower Cue quadrant also suggests that animals spend most of their time at low speeds or stopped in front of the Cue.

v. Swimming Pool Task

Place Task

Table 3.2. Post Hoc comparison of the total distance traveled in each quadrant in the Proximal Cue experiment.

*•• * iteSSl* ft	Cue	90°	180°	270°
Cue	.-tng."..)'^;..}.,'. - « „ , ·· t lit J .»* c ·"; •: ..	**	**	—
90°	**	1	•/-I —	—
180°	**	—	1 1 I· if	—
270°	—	—	—	

Figure 3.6. Mean and standard error of the average speed traveled in each quadrant. This graph demonstrates a significant effect of quadrant ($F(3,39)=12.526$; $p<0.0001$).

Proximal Cue: Average Speed

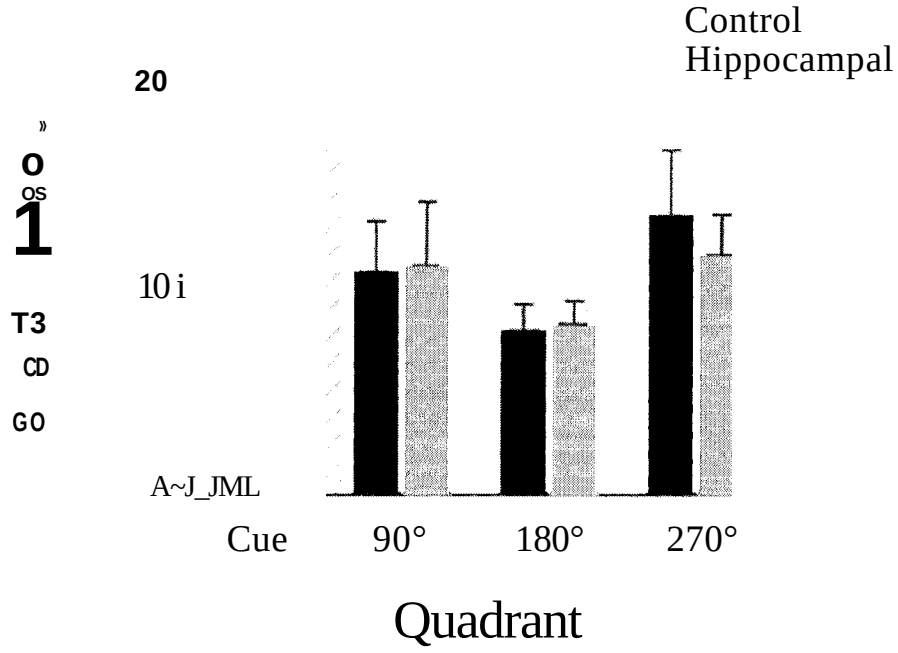


Table 3.3. Post Hoc comparison of the average speed traveled in each quadrant in the Proximal Cue experiment.

i / ft *J**	Cue	90°	180°	270°
Cue		**#	***	***
90°	***		—	—
180°	***	—		*
270°	***	—	*	

A within subjects analysis of variance revealed a significant group effect ($F(1,18)=40.156$; $p<0.001$; Fig. 3.7). There was also a significant effect of day ($F(9,162)=9.49$; $p<0.001$). There was also a significant day x group interaction ($F(9,162)=8.45$; $p<0.01$). In all examples, control animals had lower mean escape latencies. Consequently, control animals show a correct learning curve for this task while hippocampectomized rats are impaired on their learning of the hidden platform.

Matching to Place

A within subjects analysis of variance revealed a significant group effect ($F(1,8)=8.533$; $p=0.019$; Fig. 3.8). There was also a significant effect of day ($F(4,32)=4.943$; $p=0.003$). There was also a significant effect of trial ($F(1,8)=32.438$; $p<0.001$). There was a significant day x group interaction ($F(4,32)=3.90$; $p<0.05$). There was a significant day x trial interaction ($F(4,32)=5.173$; $p<0.05$). There was a significant trial x group interaction ($F(4,32)=9.216$; $p=0.016$). Finally, there was also a significant day x trial x group interaction ($F(4,128)=7.028$; $p=0.007$). In conjunction with the results from the Place Task, the Matching to Place results show the control group to be capable of learning the new platform location between the first and second trials. The hippocampectomized animals are clearly impaired on the second trial of the Matching to Place and show little or no learning.

vi. Histology

The hippocampus was completely sectioned in both control and hippocampectomized animals (Fig. 3.9). The tract from the canula caused only slight damage to the structures above the hippocampus that it passed through. All

Figure 3.7. Mean and standard error of the latency to find the hidden platform in the water maze Place Task. A within subjects analysis of variance revealed a significant day x group interaction ($F(9,162)=8.45$; $p<0.01$). In all examples, control animals had lower mean escape latencies. Consequently, control animals show a correct learning curve for this task while hippocampectomized rats are impaired on their learning of the hidden platform.

Water Maze: Place Task

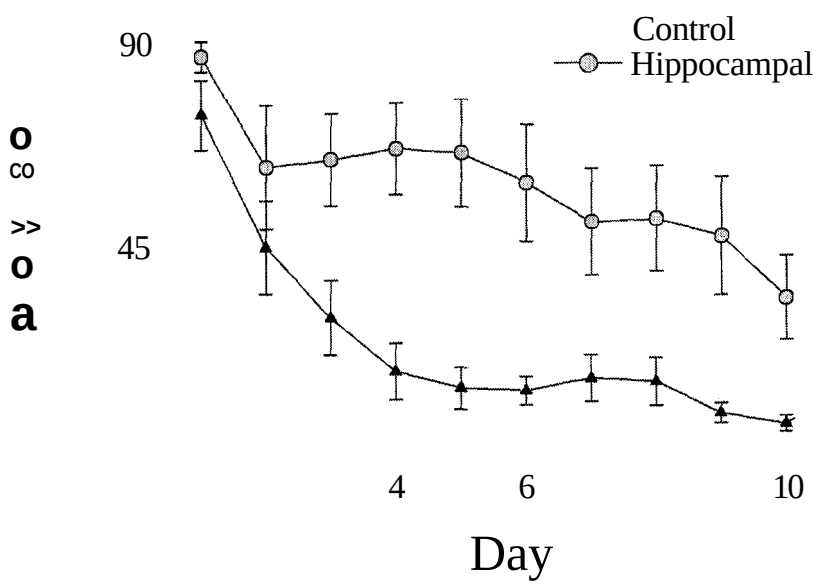


Figure 3.8. Mean and standard error of the latency to find the hidden platform in the water maze Matching to Place task. A within subjects analysis of variance revealed a significant day x trial x group interaction ($F(4,128)=7.028$; $p=0.007$).

Water Maze: Matching to Place

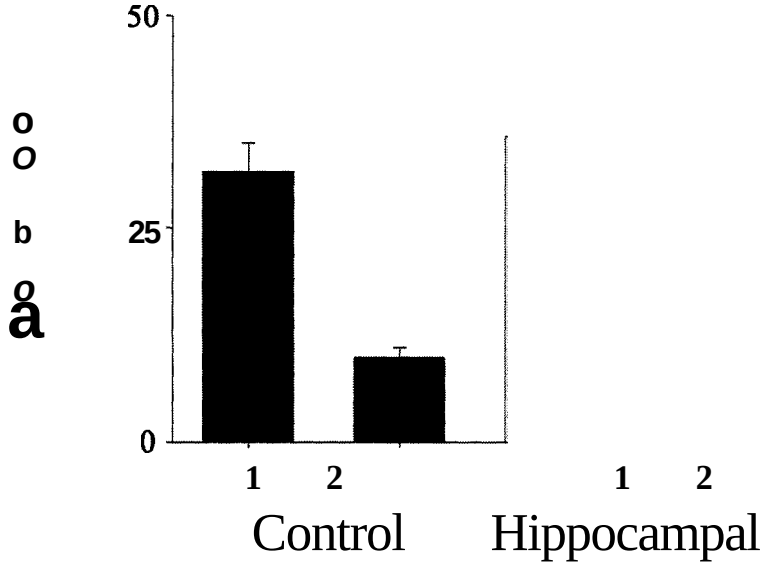


Figure 3.9. Histology of control and hippocampectomized rat brains, sectioned through the hippocampus. Figure 3.9a shows a control rat section, while Figure 3.9b shows a hippocampectomized rat brain. All hippocampectomized animals had virtually complete loss of both pyramidal and granule cells.

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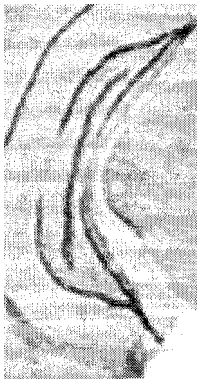


/ ^

hippocampectomized animals had virtually complete loss of both pyramidal and granule cells. Two rats had a minimal of cell survival in the dorsal portion of the hippocampus' CA3 cells. In none of the rats were structures outside of the hippocampus damaged.

IV. Discussion

An ethological examination of spatial behaviour found that proximal cues have a role in home base formation. Both control and hippocampectomized animals organized their spatial behaviours in relation to the cue with no obvious group differences. The results of the Swimming Pool task confirmed that there are deficits in spatial learning (Morris et al., 1981; Morris et al., 1982; Sutherland et al., 1983)

The design of the experiment was such that all animals experienced the proximal cue in all quadrants in a random sequence. This experiment found that both control and hippocampectomized animals followed the proximal cue on all days. Olfactory and distal cues were not used because if animals used these cues they would have been in the previous days locations where these cues were still present.

Control and hippocampectomized animals all spent more time, traveled a greater distance, and at a lower average speed in the Cue quadrant. In conjunction with the graphs showing representative paths and stops we find both groups organizing behaviour around the proximal cue and therefore the home base. Home base behaviours can thus be organized in relation to proximal cues, and this organization does not depend on the hippocampus.

That hippocampectomized rats form a home base in relation to a large proximal cue is consistent with spatial mapping theory that proposes that hippocampectomized rats

can use local cues and taxon responses to organize their behaviour (O'Keefe & Nadel, 1978). To determine whether rats can use distal cues, is the subject of the second experiment.

CHAPTER 4

EXPERIMENT 2 - ROLE OF DISTAL CUES & THE HIPPOCAMPUS

/. *Introduction*

The findings of (O'Keefe & Conway, 1978) have shown that place cells in the hippocampus organize spatial behaviour and that this behaviour is organized around cues. The previous experiment suggests that hippocampectomized animals are capable of using proximal cues to organize their behaviour. A possible explanation for these results is that the proximal cue served solely as a beacon and promoted taxon strategies for both groups. Thus, organized behaviour reported in the first experiment could be mediated by a taxon strategy.

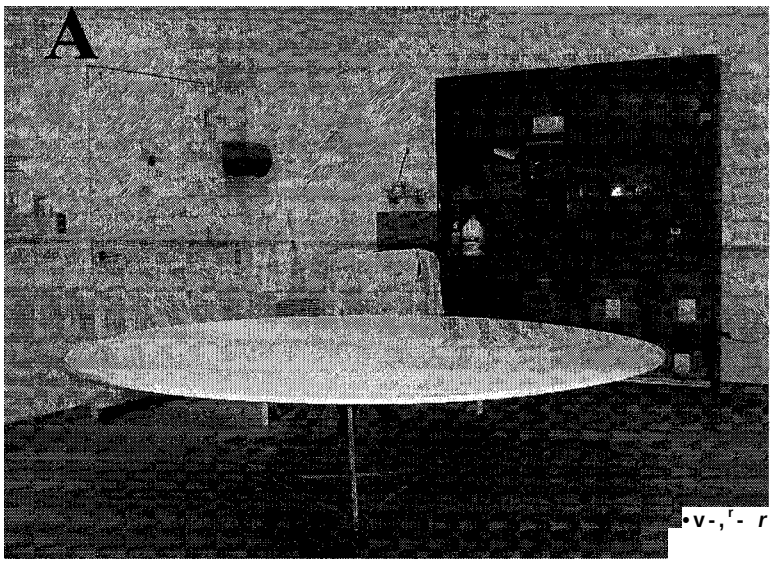
The second experiment was designed to investigate whether distal cues have a role in the formation of a home base. Control rats and rats with hippocampal lesions were tested in the same room, but distal cues were manipulated. The most prominent distal cue was a bookcase, and so for all trials this cue was present, removed, or enhanced.

//. *Methods*

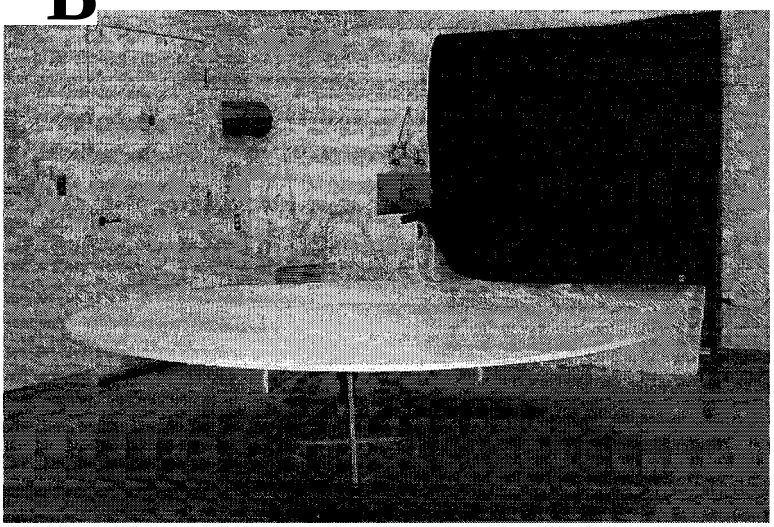
A total of 15 female Long-Evans rats were used assigned to control (n=8) and hippocampal (n=7) groups. The table that served as an experimental apparatus was divided into four equal quadrants. The division of these quadrants was such that a bookcase was positioned at the centre of the cue quadrant (Fig. 4.1). The quadrant that

Figure 4.1. Photograph of the apparatus used in the Distal Cue experiment. In experiment 2, the bookcase served as the Distal Cue. In different variations of the task, the bookcase was used alone (Fig. 4.1a), or when covered by either a dark sheet (Fig. 4.1b) or a white sheet (Fig. 4.1c).

A



B



was 270° from the book case contained a sink and a door, the only other dominant distal room cues. The testing room contained numerous cues that remained constant throughout all trials. The book case was manipulated by covering it with a dark, white or no sheet at all. Animals received two-hour trials and experienced all three conditions in a randomized manner over a three-day period. Movements were video recorded for two hours after the subjects were placed on the apparatus and then the subject was removed.

Ill, Results

i. Paths and Stops

Intrpretation of paths and stops revealed the location of the home base to be influenced by Distal Cues (Fig. 4.2 - Fig. 4.4). Animals set up their home base in front of the both the book case and dark sheet, represented by circles with diameters corresponding to the amount of time spent at that location. Typical home base behaviours were also seen at these locations. Path analysis shows that animals made trips to and from the home base. When returning to the home base, animals took trips that were more direct when compared to the trips outward from the home base.

ii. Quadrant time

Dark Sheet

A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect quadrant ($F(3,39)=43.762$; $p<0.0001$; Fig. 4.5).

Figure 4.2. Paths and stops with the Dark Sheet Cue, for typical control and hippocampectomized rats. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. Stops are represented by circles, with diameters corresponding to the amount of time spent at that location. This pattern of behaviour suggests that animals set up their home base in front of the Distal Dark Sheet Cue.

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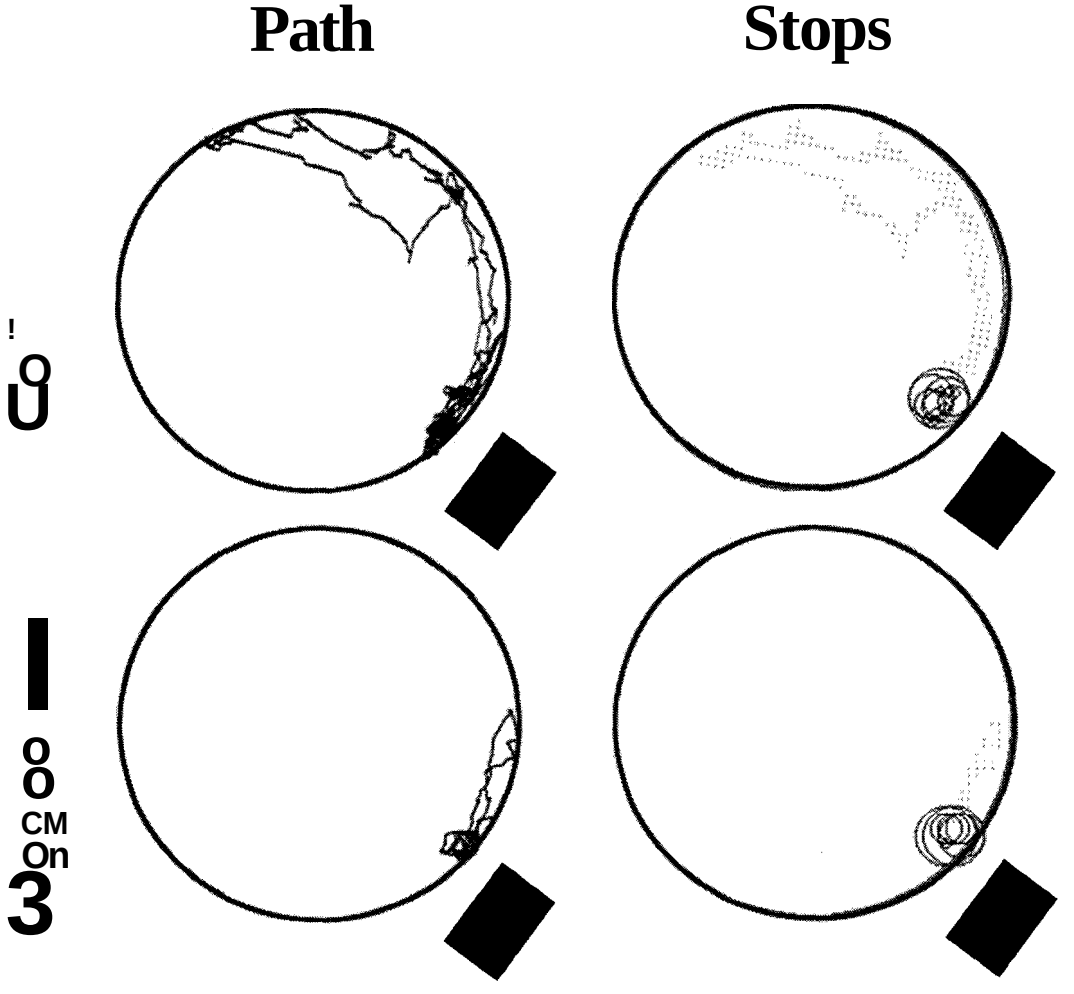


Figure 4.3. Paths and stops with the Bookcase, for typical control and hippocampectomized rats. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. Stops are represented by circles, with diameters corresponding to the amount of time spent at that location. This pattern of behaviour suggests that animals set up their home base in front of the Distal Bookcase Cue.

Bookcase

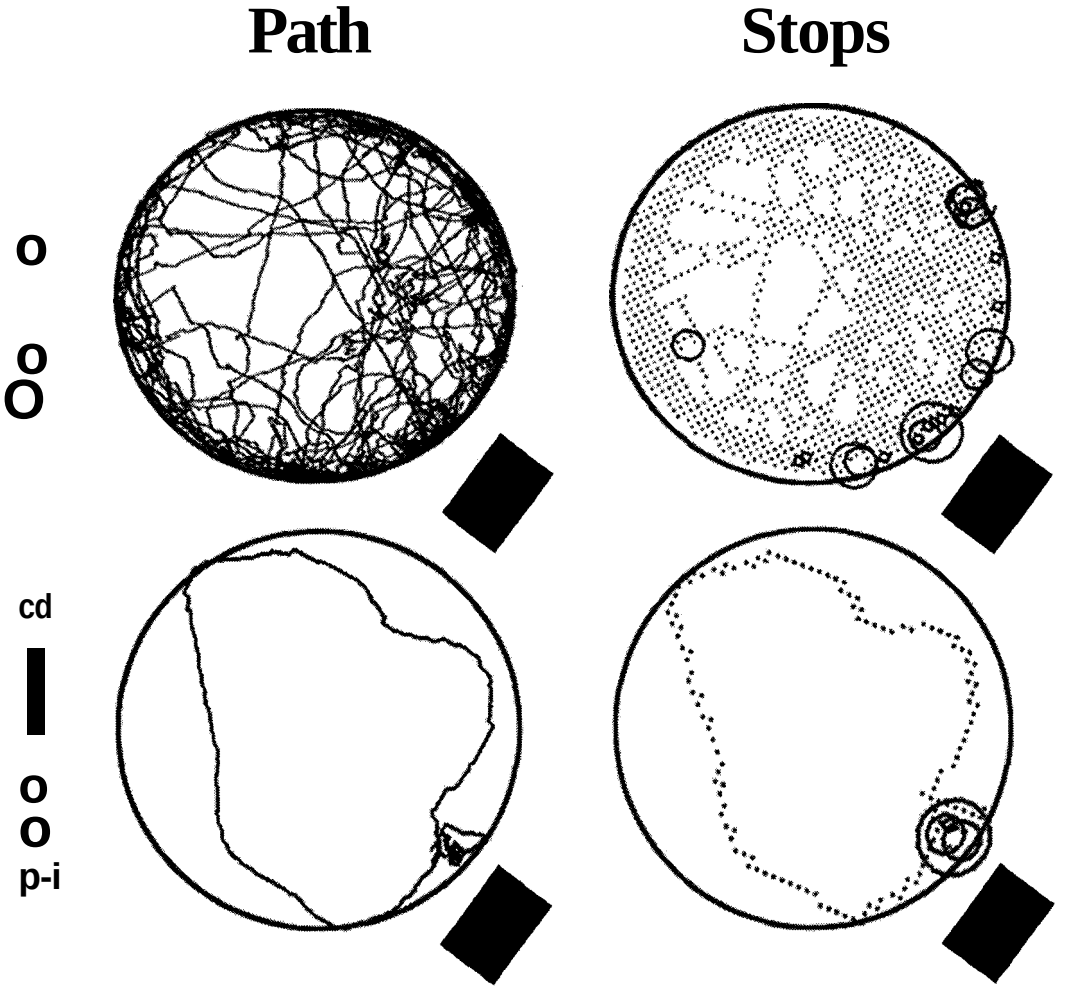


Figure 4.4. Paths and stops with the White Sheet, for typical control and hippocampectomized rats. Examination of the paths and stops revealed the location of the home base was not substantially influenced by the White Sheet Cue.

White Sheet

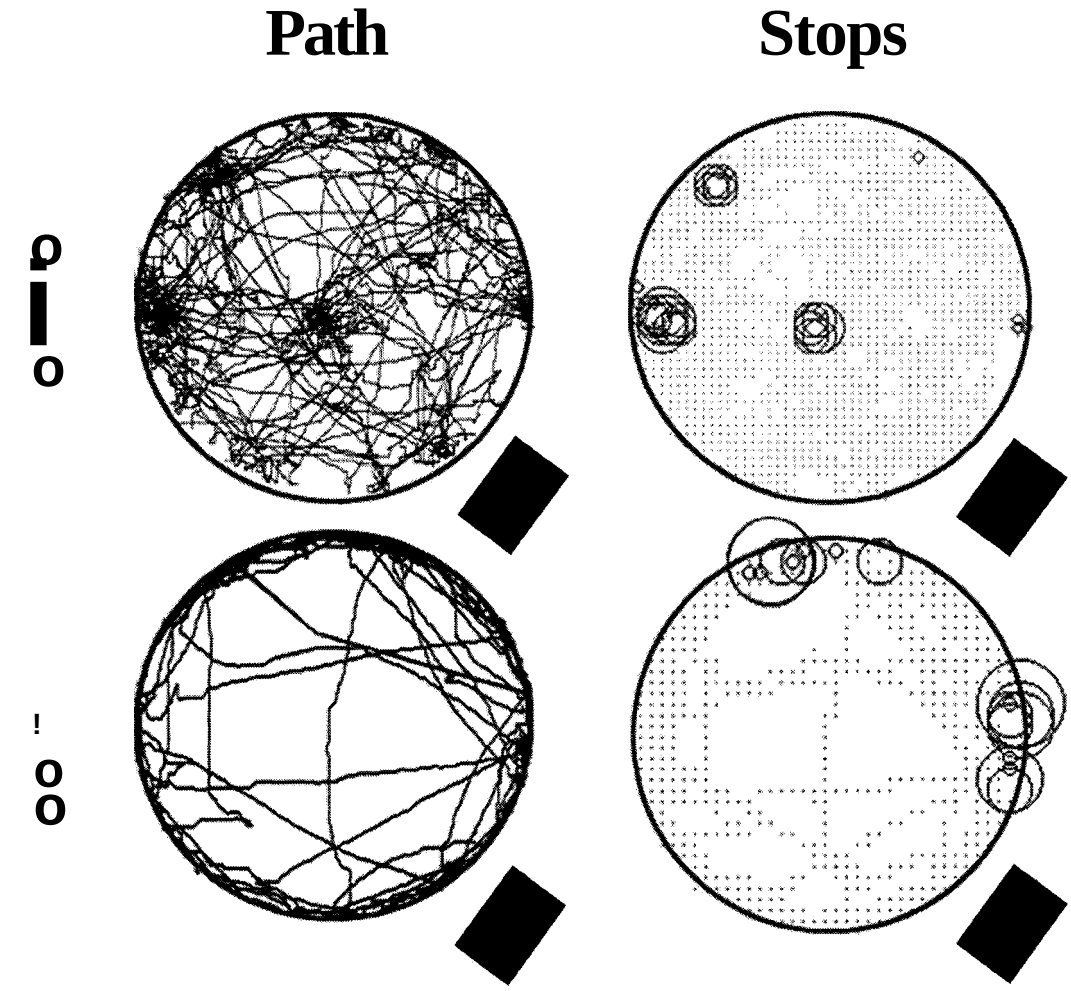
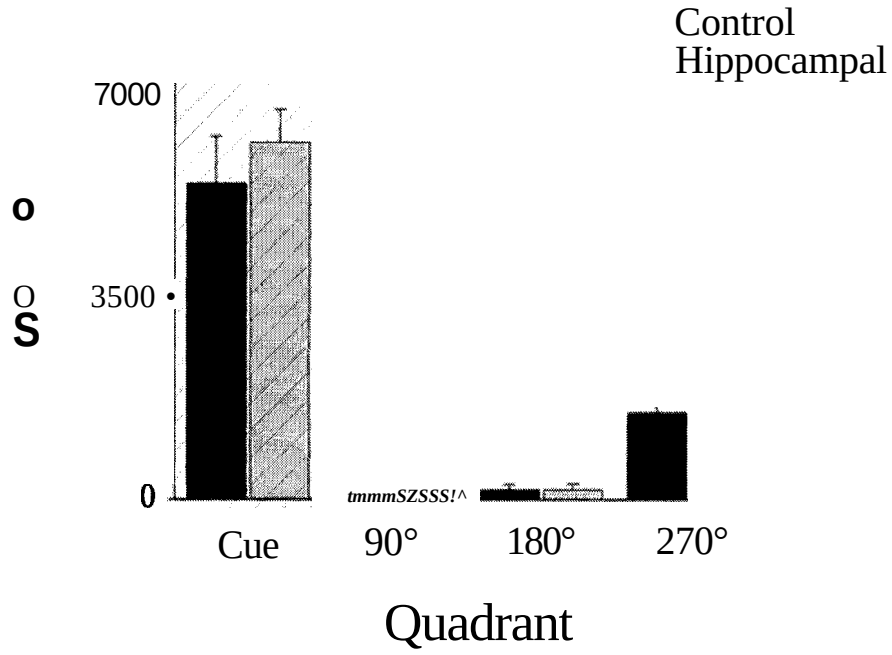


Figure 4.5. Mean and standard error of the total time spent in each quadrant in the Dark Sheet experiment. This graph demonstrates a significant effect of quadrant ($F(3,39)=43.762$; $p<0.0001$).

Distal Cue - Dark Sheet: Time



There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparisons (Bonferroni LSD; $p<0.0001$; Table 4.1) revealed that the quadrant with the Dark Sheet was where animals spent the majority of their time. The quadrant effect was only seen in the Dark Sheet quadrant and animals had a mean of total time 5830.679 seconds (±508.343) per day. These results demonstrate that both control and hippocampectomized animals spend most of their time in the distal cue quadrant.

Book Case

A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect quadrant ($F(3,39)=9.479$; $p<0.0001$; Fig. 4.6). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparisions (Bonferroni LSD; $p<0.0001$; Table 4.2) revealed that the quadrant with the Book Case was where animals spent the majority of their time. The quadrant effect was also seen in the quadrant with that contained the door and the sink and was 270° from the cue (Bonferroni LSD; $p<0.0001$). The quadrant effect was only seen in the Dark Sheet quadrant and animals had a mean of total time 5830.679 seconds (±508.343) per day. These results demonstrate that both control and hippocampectomized animals spend most of their time in the quadrant containing the bookcase.

White Sheet

A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect of quadrant ($F(3,39)=3.622$; $p=0.02$; Fig. 4.7).

Table 4.1. Post Hoc comparison of the total time spent in each quadrant with the Dark Sheet.

"" *"-CT« "- Cue	90°	180°	270°
Cue 1 ***		***	***
90° ***	1 1 _		—
180° ***			—
270° ***		"~^–	

Figure 4.6. Mean and standard error of the total time spent in each quadrant in the Bookcase experiment. This graph demonstrates a significant effect of quadrant ($F(3,39)=9.479$; $p<0.0001$).

Distal Cue - Book Case: Time

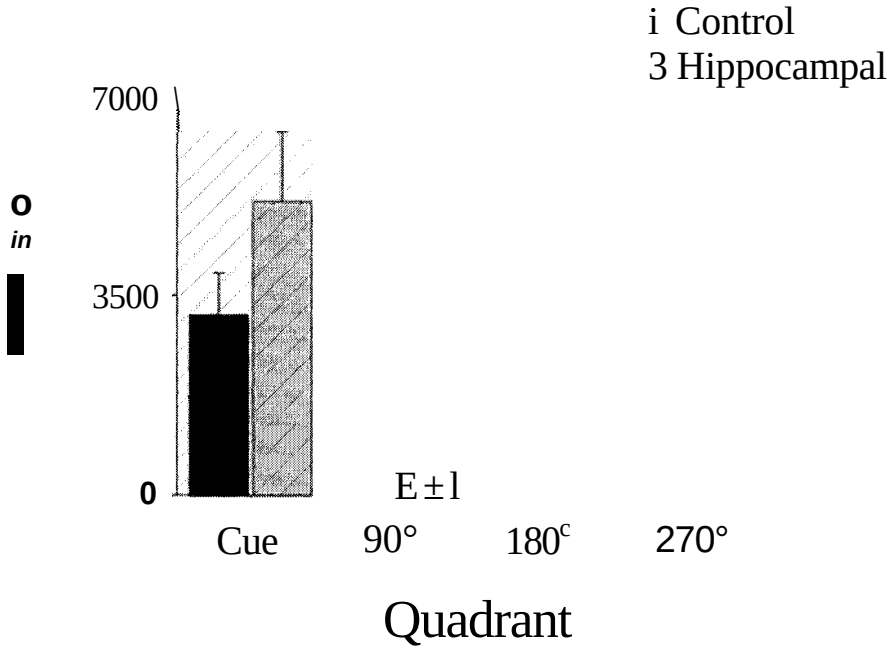
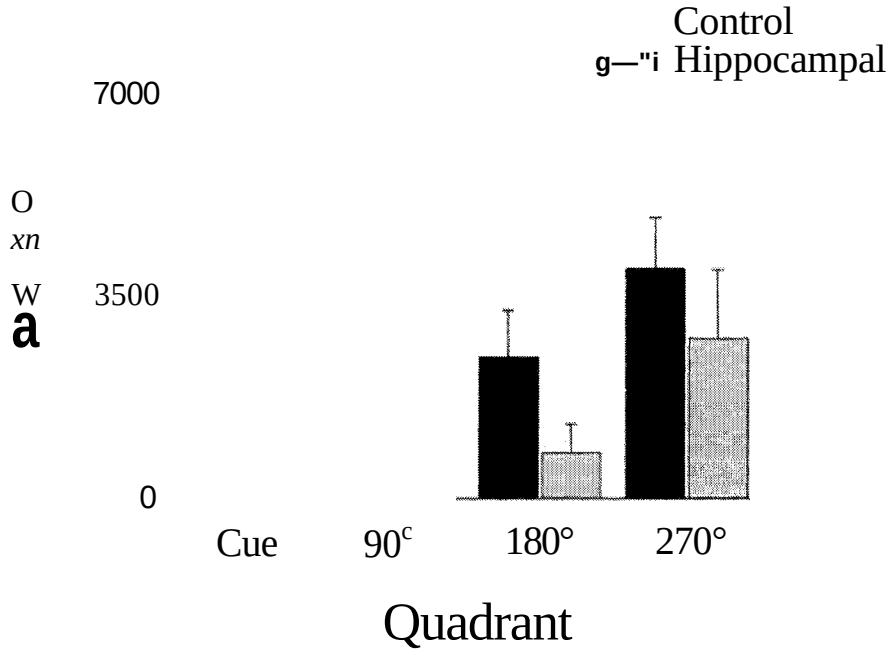


Table 4.2. Post Hoc comparison of the total time spent in each quadrant in the Bookcase experiment.

	Cue	90°	180°	270°
Cue		***	*	**
90°	***		*	**
180°	*	*		—
270°	**	**		

Figure 4.7. Mean and standard error of the total time spent in each quadrant in the White Sheet experiment. This graph demonstrates a significant effect quadrant ($F(3,39)=3.622$; $p=0.02$).

Distal Cue - White Sheet: Time



There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparisons (Bonferroni LSD; $p=0.034$; Table 4.3) revealed that the 270°quadrant containing the door or sink was where animals spent the majority of their time. Coupled with an examination of the animals' paths and stops, the animals stopped in the quadrant containing the door and sink.

iii. Cue Salience and Preference

An analysis of the effect of the cues saliency, that is the difference between the high saliency of the dark sheet and the low saliency of the white sheet, showed no group effect between control and hippocampectomized rats (Fig. 4.8, Table 4.4). There was a significant effect of the cue salience itself ($F(2,26)=17.213$; $p<0.0001$). The book case and the dark sheet did not differ in the amount of time that animals spent in front of them, but the white sheet was significantly less influential (Bonferroni LSD; $p<0.001$).

The amount of time spent in a quadrant is one of the most likely indicators of a home base. An analysis of the quadrant that animals preferred for all three conditions was calculated by subtracting the amount of time spent in the quadrant where the condition was manipulated from all other quadrants and averaging this by the total number of quadrants. The preference score was calculated by the following formula:

$$\text{Cue Preference} = ((\text{time}(\text{Cue-90°})) + (\text{time}(\text{Cue-180})) + (\text{time}(\text{Cue-270})))/3$$

A student's t- test revealed that animals preferred the Dark Sheet condition over both the Book Case ($p=0.024$) and the White Sheet ($p<0.0001$; Fig. 4.9, Table 4.5).

Table 4.3. Post Hoc comparison of the total time spent in each quadrant in the White Sheet experiment.

**J <^Sf-*VJ PUP		90°	180°	270°
Cue , 4				*
90°	–	1 –		*
180°	–	–		–
270°	*	*	–	

Figure 4.8. The difference between the high saliency of the dark sheet and the low saliency of the white sheet, showed no group effect between control and hippocampectomized rats. There was a significant effect of the cue salience itself ($F(2,26)=17.213$; $p<0.0001$).

Distal Cue - Cue Salience

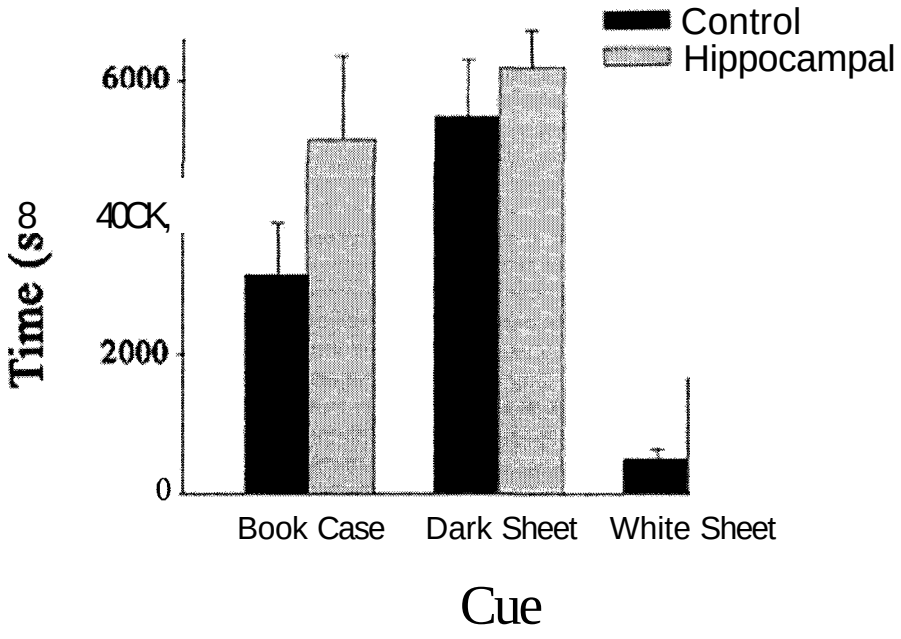


Table 4.4. Post Hoc comparison of Cue Salience in the Distal Cue experiment.

*	I) II k Sheet	Book Case	White Sheet
Dark Sheet			**
Book Case			H« *
White Sheet	**	##	

Figure 4.9. Mean and standard error of Cue Preference during the Distal Cue experiment. A student's T test revealed that animals preferred the Dark Sheet condition over both the Book Case ($p=0.024$) and the White Sheet ($p<0.0001$). Animals also preferred the Book Case over the White Sheet ($p=0.028$).

Distal Cue - Cue Preference: Time

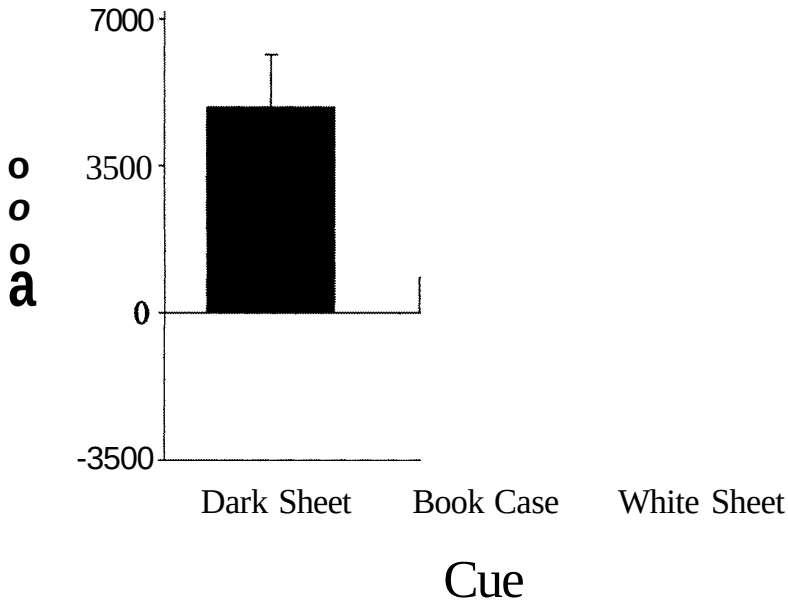


Table 4.5. Post Hoc comparison of Cue Preference in the Distal Cue experiment.

9 - <-	Dark Sheet	Book Case	White Sheet
Dark Sheet		x	***
Book Case	*	<	*
White Sheet	xxx	x	r -

Animals also preferred the Book Case to the White Sheet ($p=0.028$). These results suggest that animals prefer distal cues that offer them information that is readily contrasted from the environment.

iv. Distal Cue Distance

The distance that was traveled in the distal quadrant was also compared across all animals using a student's t-test. There was no significant difference between the distance traveled in the Dark Sheet quadrant and Book Case Quadrant (Fig. 4.10; Table 4.6). The distances traveled were significantly lower in the Dark Sheet quadrant when compared to the White Sheet ($p=0.026$). Animals from both groups traveled further and spent less time in the distal cue quadrant when the White Sheet was placed over the Book Case.

v. Distal Cue Speed

The speeds at which the animals traveled in the distal quadrant was also compared across all animals using a student's t-test. There was no significant difference between the speeds traveled in the Dark Sheet quadrant and Book Case Quadrant (Fig. 4.11; Table 4.7). The speeds traveled were significantly lower in the Dark Sheet ($p<0.001$) and Book Case quadrant when compared to the White Sheet ($p=0.002$). Animals from both groups traveled more slowly and spent less time in the distal cue quadrant when the White Sheet was placed over the bookcase. The Dark Sheet and Book Case significantly decrease the

Figure 4.10. Mean and standard error of total distance traveled in each of the three Distal Cue conditions. The distances traveled were significantly lower in the Dark Sheet quadrant when compared to the White Sheet ($p=0.026$).

Distal Cue - Cue Comparison: Distance

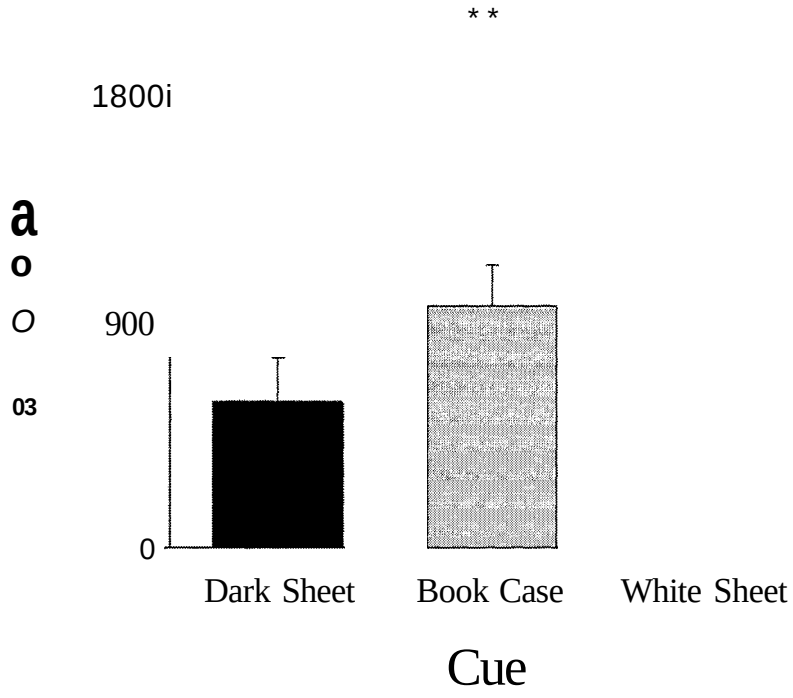
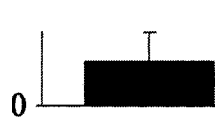


Table 4.6. Post Hoc comparison of total distance traveled in each of the three Distal Cue conditions.

1. „., Z	Dark Sheet	Book Case	White Sheet
Dark Sheet \, „- '. v- -... :			*
Book Case	—		
White Sheet	*		

Figure 4.11. Mean and standard error of the average speed traveled in each of the three Distal Cue conditions. The average speed traveled was significantly lower in the Dark Sheet ($p<0.001$) and Book Case quadrant when compared to the White Sheet ($p=0.002$).

Distal Cue - Cue Comparison: Speed



Dark Sheet Book Case White Sheet

Cue

Table 4.7. Post Hoc comparison of average speed traveled in each of the three Distal Cue conditions.

*S F * 1 \	Dark Sheet	Book Case	White Sheet
Dark Sheet			***
Book Case	—		
White Sheet	***		

speed animals traveled in that quadrant presumably because animals are largely stopped when in those quadrants during those conditions.

IV. Discussion

An examination of spatial behaviour, in an environment in which distal cues were manipulated indicated distal cues have a role in home base formation. Both control and hippocampectomized animals organized their spatial environment using the distal cues and there were no obvious group differences. The present study is definitive in indicating that distal cues organize the formation of the home base and corresponding behaviours.

The present study does result in a striking contrast with the literature concerning the role of the hippocampus in spatial learning and organization. Spatial mapping theory proposes that hippocampectomized rats will not use distal cues (O'Keefe & Nadel, 1978). Here there were no differences between control and hippocampectomized rats on any of the measures. Controls and hippocampectomized animals all spent more time, traveled a greater distance, and at a lower average speed in the distal cue quadrant unless it was masked by the White Sheet. In conjunction with the graphs showing representative paths and stops we find both groups organizing behaviour around the distal cues and therefore the home base.

The results of this experiment and the first experiment show that both control and hippocampectomized animals organize their home base behaviour in response to both distal and proximal cues. It is possible, however, that hippocampectomized rats use other salient and local cues to organize their behaviour. The possibility of self-movement and olfactory cues organizing home base behaviour was examined in Experiment 3.

CHAPTER 5
EXPERIMENT 3 - ROLE OF SELF-MOVEMENT AND OLFACTORY CUES

/. *Introduction*

The previous experiments provide results suggesting both proximal and distal cues are capable of controlling the location of the home base formation in both control and hippocampectomized animals. The role of less salient cues, such as self-movement and olfaction, are less certain. Hill & Best (1981) report that place cells do not disappear when visible cues are unavailable. They also found that rotating the apparatus caused a similar rotation in hippocampal place fields. This suggest that olfactory cues can maintain place fields and create them (Hill & Best, 1981).

Whishaw, Hines, & Wallace (2001) suggest that the hippocampus plays a critical role in the processing self-movement cues. The navigational strategy using self-movement cues is called dead reckoning. The calculation of distance from a starting position to the goal is integrated with time to give a relative spatial location.

The present experiment was designed to investigate whether self-movement and olfactory cues have a role in the formation of a home base. In conjunction with this, a group of animals with hippocampal lesions also serves to investigate the necessity of the hippocampus in home base formation in total darkness. Control and hippocampectomized groups are compared to animals with olfactory bulb lesions for their ability to form a home base in total darkness.

//. *Methods*

i. Animals

A total of 16 female Long-Evans rats were assigned to a control (n=6) hippocampal (n=5), and olfactory bulbectomized (n=5) group. All rats were housed with at least one other rat in plexi-glass cages. The room in which animals were house had a constant room temperature between 20-21°C with a 12 hr light/dark cycle. Rats were fed with Lab Diet Laboratory Rodent Pellets in their home cage and allowed free access to both food and water.

ii. Olfactory Bulbectomy Surgeries

Rats were anesthetized with a mixture of isofluorane and oxygen (4% with 21 per minute of oxygen and 2% after a surgical plane was established.) An incision was made in the scalp and the periosteum to expose the cranium. A dental burr was used to drill small holes in the skull directly above the olfactory bulbs. The dura in this area was removed and the underlying tissue was removed using a glass pipette and suction pump to create suction lesion. A visual examination of the lesion area during the surgeries guaranteed complete lesions. Animals also received the opiate bupenorhpine (10 mg/kg i.p.) to alleviate pain associated with the procedure. Control animals did not undergo any surgical procedure. Animals were given two weeks to recover after the surgery before behavioural testing began.

iii. Apparatus

Foraging table: the apparatus was a wooden circular table without walls measuring 244 cm in diameter. The table was cleaned with both an antiseptic solution and a dilute ammonia solution to remove any contamination and all odor cues between the testing of each rat. All behaviours were video recorded from above using a Sony HI-8 infrared camera that was mounted perpendicular to the table. Two radios were placed in opposing corners of the room and controlled for any possible auditory cue use. All animals were given a single 2 hour exploratory session in total darkness.

///. *Results*

i. Paths and Stops

Interpretation of the paths and stops revealed the location that control and bulbectomized rats formed home bases (Fig. 5.1). Typical home base behaviours were also seen at these locations. Hippocampectomized animals' stops appear to be more random and relegated to the periphery of the table. Path analysis shows that control and bulbectomized animals made trips to and from the home base area. Hippocampectomized animals' paths are largely on the periphery of the table and lack readily observable organization.

ii. Quadrant time

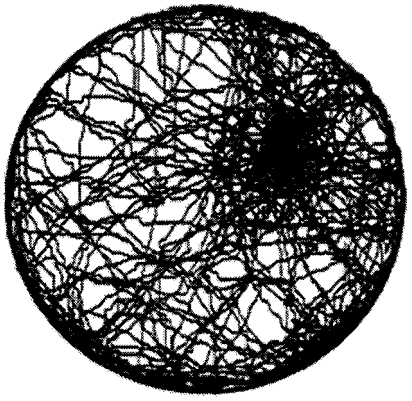
A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect ($F(3,39)=6.775$; $p<0.001$; Fig. 5.2). There was also

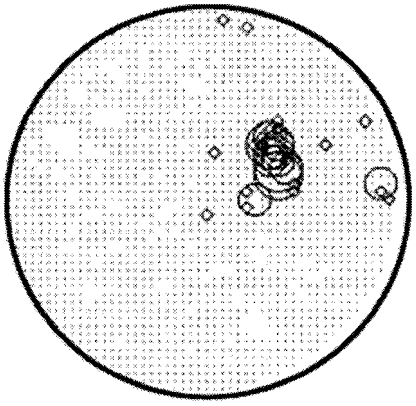
Figure 5.1. Paths and stops in total darkness, for typical control, hippocampectomized and bulbectomized rats. Control and bulbectomized animals set up their home base in a distinct area as represented by circles with diameters corresponding to the amount of time spent at that location. Hippocampectomized animals' stops appear to be more random and relegated to the periphery of the table. Path analysis shows that control and bulbectomized animals make trips to and from the home base area. Hippocampectomized animals' paths are largely on the periphery of the table and lack readily observable organization.

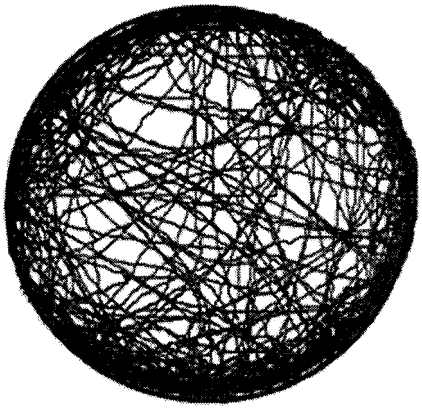
Total Darkness

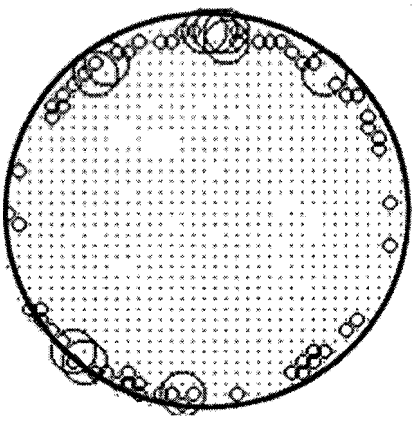
Path

Stops









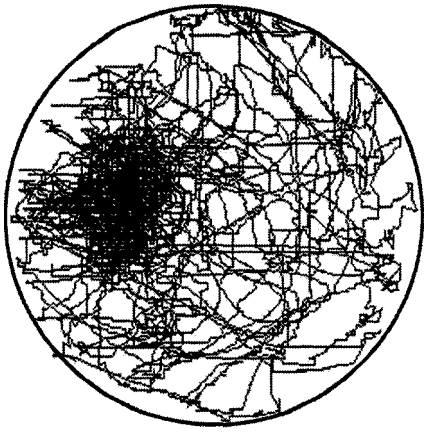
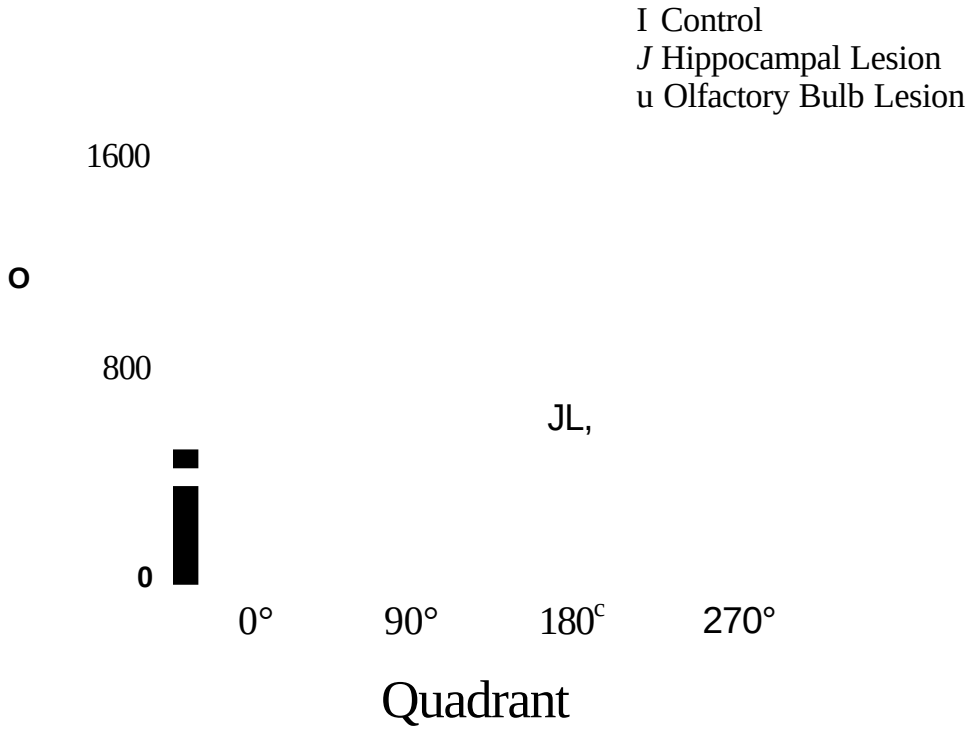


Figure 5.2. Mean and standard error of the total time spent in each quadrant in total darkness. Analysis of quadrant time revealed a significant effect of quadrant ($F(3,39)=6.775$; $p<0.001$). There was also a significant quadrant x group effect ($F(6,39)=9.268$; $p<0.0001$).

Total Darkness: Time



a significant quadrant x group effect ($F(6,39)=9.268$; $p<0.0001$; Table 5.1). Subsequent multiple comparison (Bonferroni LSD; $p<0.01$) revealed that the 270° quadrant was where the quadrant effect was seen and animals had a mean of total time 2716.2 seconds (±258.174). Coupled with an examination of the animals' paths and stops, we further see that only control animals prefer a specific quadrant.

iii. Average Number of Stops

The average numbers of stops that animals made were compared across all animals using a student's T test. There was a significant difference in the average number of stops between all groups (Fig. 5.3; Table 5.2). The control animals had 29.8 average stops (±6.05) per trial and differed significantly from both the hippocampectomized ($p=0.002$) and the bulbectomized ($p=0.002$) groups. The hippocampectomized group had 58.83 average stops (±14.26) per trial also differed significantly from the bulbectomized group ($p<0.0001$). The bulbectomized group had 14.2 average stops (±4.97) per trial. These results show that hippocampal animals stopping more than twice as much as any other group.

iv. Stop Dispersion

The average dispersion indexes of animals were compared across all animals using a student's T test. There was a significant difference in the dispersion of stops between the hippocampectomized groups and all others (Fig. 5.4; Table 5.3). The hippocampectomized animals had an average stop dispersion index of 129.84 (±22.44

Table 5.1. Post Hoc comparison of the total time spent in each quadrant in total darkness.

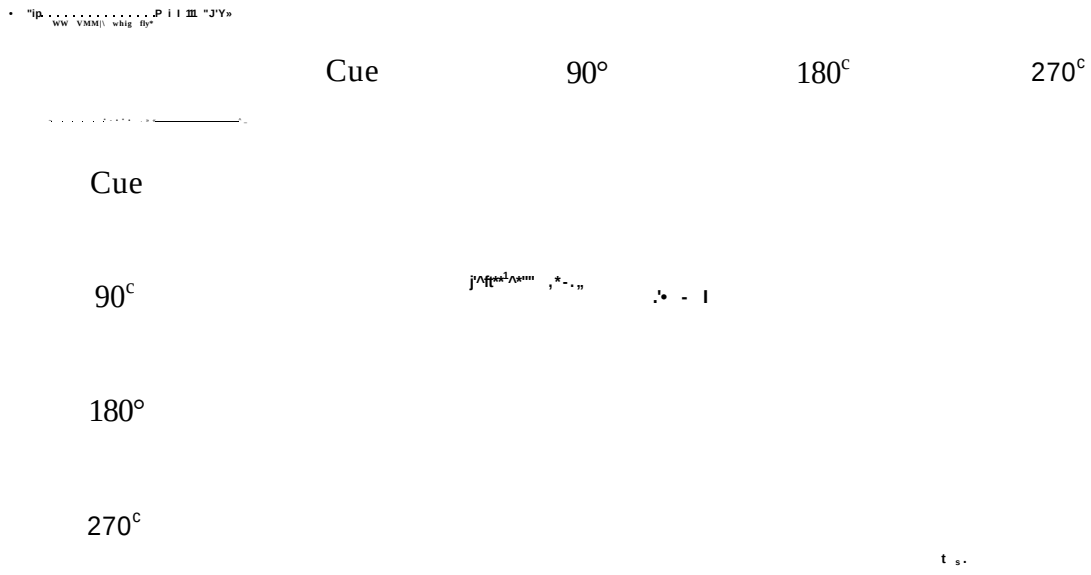

Cue
90°
180°
270°
Cue
90°
180°
270°

Figure 5.3. Mean and standard error of the average number of stops in total darkness in control, hippocamectomized and bulbectomized rats. There was a significant difference in the average number of stops between all groups.

Total Darkness: Number of Stops

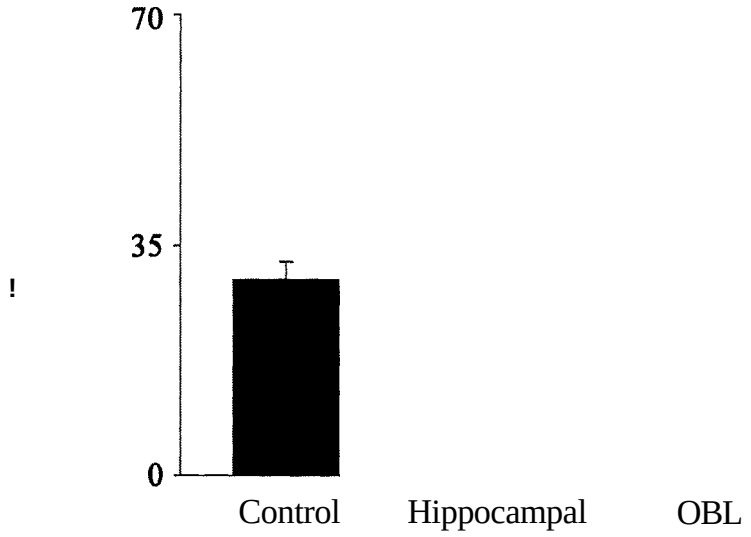


Table 5.2. Post Hoc comparison of the total number of stops in total darkness in control, hippocampectomized and bulbectomized rats.

	Control	Hpc	OBL
Control	*	**	**
Hpc	**	" , : . * –' r > - # * *	
OBL	**		

Figure 5.4. Mean and standard error of the stop dispersion index in control, hippocampectomized and bulbectomized rats. There was a significant difference in the dispersion of stops between the hippocampectomized groups and all others.

Total Darkenss: Dispersion

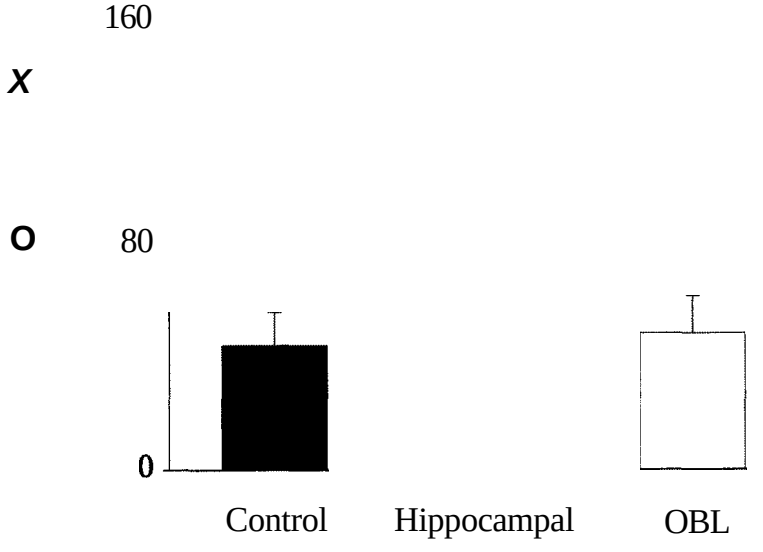


***Table** 5.3.* Post Hoc comparison of the stop dispersion index in control, hippocampectomized and bulbectomized rats.

*T~ * -a	Control	Hpc	OBL
Control	• w -j • . . . . . -;	**	—
Hpc	**		*
OBL			

per trial and differed significantly from both the control (p=0.009) and the bulbectomized (p=0.013) groups. The control group had an average dispersion index of 43.48 average stops (±11.61) per trial and did not differ significantly from the bulbectomized. The bulbectomized group had an average dispersion index of 47.72 average stops (±13.11) per trial. In summary, only the hippocampectomized animals had dispersed stops.

IV. Discussion

An examination of the home base behaviour confirmed that hippocampectomized rats did not form a home base, whereas both control and bulbectomized rats did when visual information was unavailable. The present study further suggests that self-movement cues rather than olfactory cues are used for the home base formation in total darkness.

The quadrant effect was seen only in the 270° quadrant, and is surprising. There is a possible explanation for the control group's choice of the 270°quadrant. This quadrant is closest to the door of the room, so possibly the animals were responding to the direction from which they entered the room or to the last location that they saw light.

Although bulbectomized animals would also have access to self movement cues, and the memory of the door and light, it is puzzling why they formed home bases, but not in relation to the door quadrant.

CHAPTER 6
EXPERIMENT 4 - PROXIMAL CUE REMOVAL AND SPATIAL LEARNING

/. *Introduction*

One finding about cue use in place cell experiments is that a single cue's removal does not disrupt the place field representation of that environment (J. O'Keefe & Conway, 1978). This finding suggested that a constellation of cues is responsible for the stability of place fields. However, when animals are tested in a circular environment when a single proximal cue is removed, place fields remained intact but rotated in a random fashion (Muller & Kubie, 1987).

In addition, Hetherington and Shapiro (1997) find that although removing a single cue does not disrupt place fields, subtle changes are seen in place field firing, eg., place cell firing decreased. These results suggest that the hippocampus is necessary to optimize spatial information and required for flexible representations of the environment

The present experiments asks whether the hippocampus is necessary for home base behaviour when cues are manipulated. Rats were first exposed to an environment with a salient proximal cue, and then the cue was removed.

//. *Methods*

i. Proximal Cue Probe

A total of 12 female Long-Evans rats were used assigned to a control (n=6) and hippocampectomized (n=6) groups. In the Proximal Cue condition, a large black box (80 x 47 x 42 cm) was placed 30 cm back from the edge of the table. The bottom of the box

was 66 cm of the floor or roughly at the same height as the table. Animals were placed in the centre of the table facing randomized compass positions. Trials were 30 minutes in duration and subjects received one trial per day over a four-day period. The table was divided into four imaginary quadrants with the cue in one quadrant. Animals were tested for four days with the cue in a constant quadrant and on the fifth day (probe day) the cue was removed. The protocol of this experiment differs from the first experiment in that the cue is not moved to the different quandrants over the first four days and that it is completely removed on the fifth/probe day. Results show the measures from the probe day.

///. *Results*

i. Time at Cue per Day

There were no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect for the time spent at the cue (or old cue location) per day (Fig. 6.1). These results show that both groups are flexible in the cues that they use to form the home base location.

ii. Paths and Stops

Examination of the paths and stops revealed the location of the home base to be in the location of the Proximal cue even when it had been removed (Fig. 6.2). All animals set up their home base in front of the location where the Proximal cue had previously been. Typical home base behaviours were also seen at home base location. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct as compared to the outward trips.

Figure 6.1. Mean and standard error of the time spent at the cue per day in the Proximal Probe experiment. There were no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect for the time spent at the cue.

Proximal Cue Probe: Time at cue per day

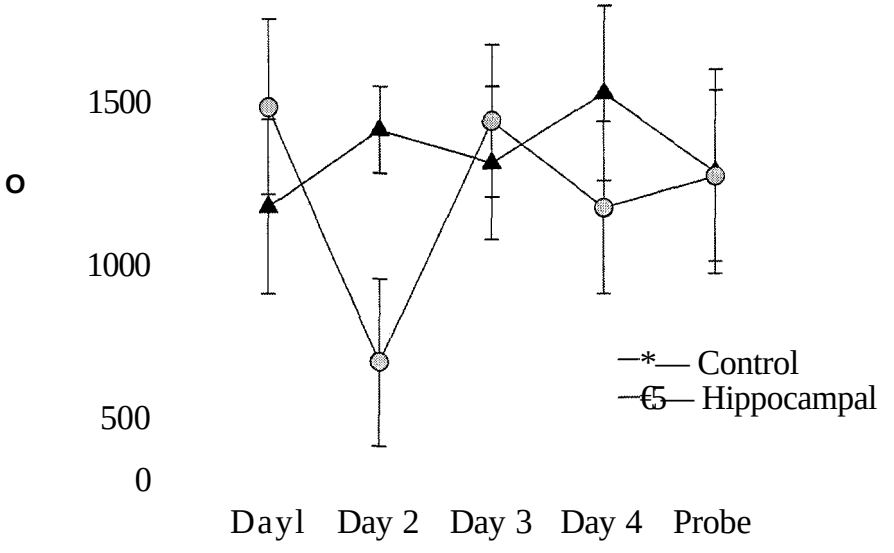
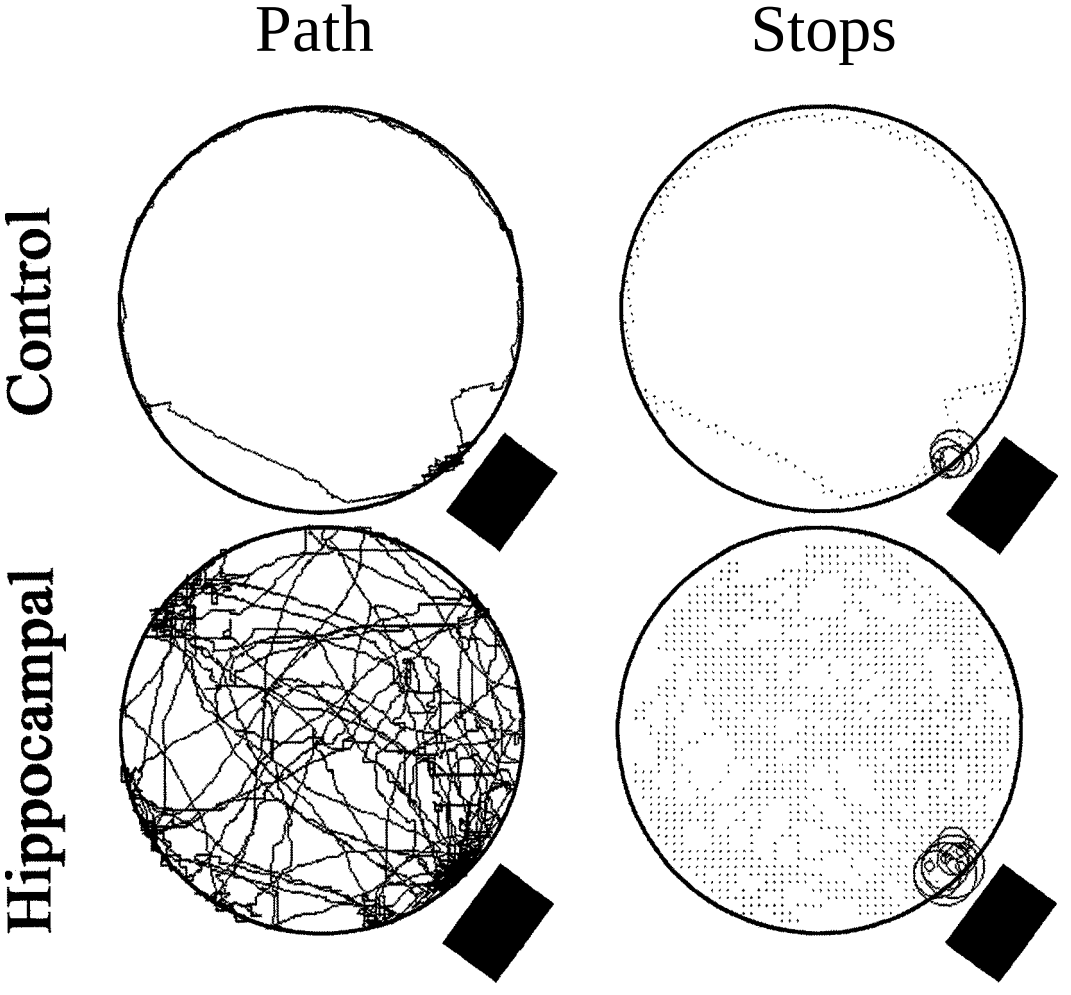


Figure 6.2. Paths and stops for typical control and hippocampectomized rats in the Proximal Probe experiment. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. Stops are represented by circles, with diameters corresponding to the amount of time spent at that location. This pattern of behaviour suggests that animals set up their home base in front of the Proximal Cue.

Proximal Cue Probe



iii. Quadrant Time

A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect quadrant ($F(3,30)=12.169$; $p<0.0001$; Fig. 6.3). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparision (Bonferroni LSD; $p<0.0001$; Table 6.1) revealed that quadrant that previously contained the cue before the probe was where animals spent the majority of their time. The quadrant effect was only seen in the Cue quadrant and animals had a mean of total time 1256.30 seconds (±204.35) on the probe day. These results demonstrate that both control and hippocampectomized animals spend most of their time in the old cue quadrant. Coupled with an examination of the animals' paths and stops, we further see that animals are stopped in front of the old proximal cue location.

iv. Quadrant Distance

A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed no significant quadrant, group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect (Fig. 6.4). A subsequent multiple comparison (Bonferroni LSD; $p=0.035$; Table 6.2) revealed that the only difference in distance was between the old Cue location and the 270° quadrant. Animals had a mean of total distance in the Cue quadrant of 978.22 cm (±283.97) per day. These results also show that both groups, control and hippocampectomized animals, traveled significantly less in the old Cue quadrant when compared to the 270° quadrant. Coupled with the

Figure 6.3. Mean and standard error of the total time spent in each quadrant. This graph demonstrates a significant effect quadrant ($F(3,30)=12.169$; $p<0.0001$).

Proximal Cue Probe: Quadrant Time

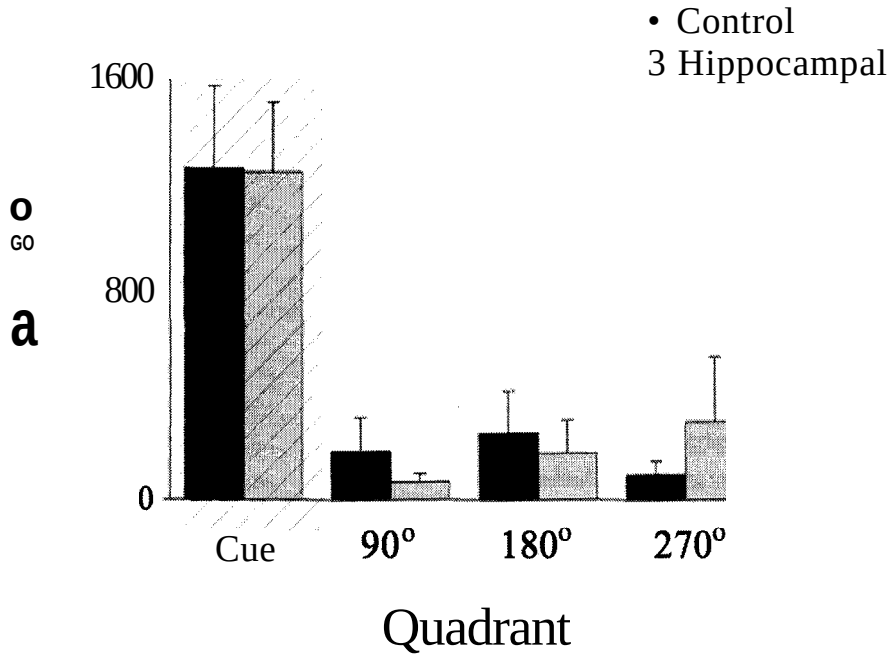


Table 6.1. Post Hoc comparison of the total time spent in each quadrant in the Proximal Probe experiment.

. s». * .. '. Cue	Cue	90°	180°	270°
Cue			**	**
90°	***			—
180°	**		1- *	
270°	**		—	

Figure 6.4. Mean and standard error of the total distance traveled in each quadrant in the Proximal Probe experiment. A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed no significant quadrant, group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect.

Proximal Cue Probe: Distance

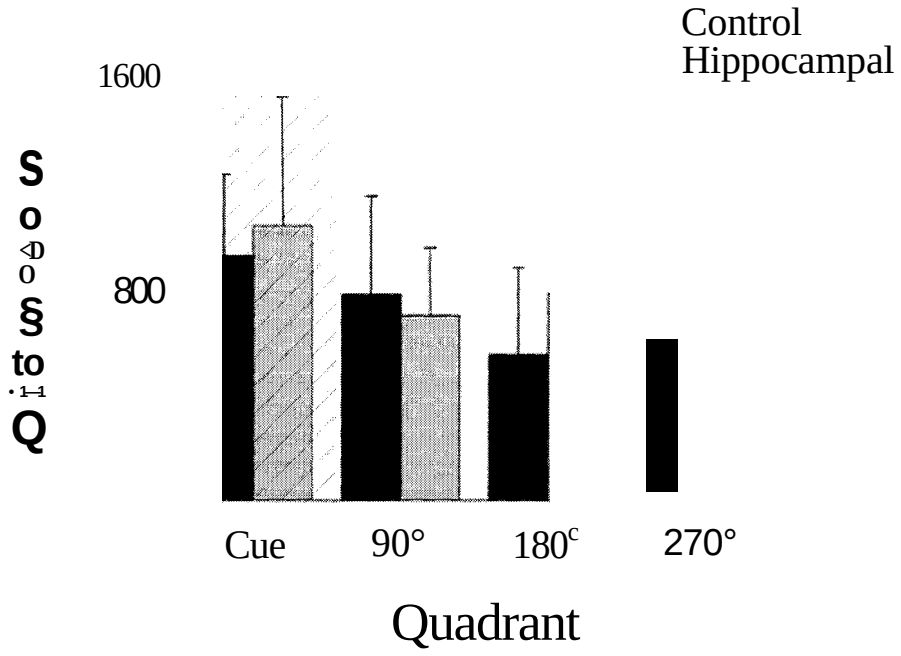


Table 6.2. Post Hoc comparison of the total distance traveled in each quadrant in the Proximal Probe experiment.

	Cue	90°	180°	270°
Cue	* *	—	—	*
90°				—
180°				-
270°	*	—		

significant amount of time that animals spent in the cue quadrant, we see that animals are largely immobile in the old Cue quadrant.

v. Quadrant Speed

A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant quadrant effect ($F(3,30)=3.51$; $p<0.027$; Fig. 6.5). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparison (Bonferroni LSD; $p<0.05$; Table 6.3) revealed that the Cue quadrant was where animals traveled at the slowest speeds when compared to the 90°and 270° quadrant. Animals had a mean speed in the Cue quadrant of 1.803 cm/second (±0.739) per day. The significantly lower speed in the Cue quadrant also suggests that animals spend most of their time at low speeds or stopped in front of the old cue location.

IV. Discussion

Both control and hippocampectomized animals organized their spatial environment using the proximal cues. When the cue was removed, both groups were able to locate the previous location of the home base with no obvious group differences. The present study indicates that hippocampectomized animals are capable of learning the location of the home base in relation to distal cues.

The present study contrasts with the argument that the hippocampus is necessary for spatial learning (Morris, 1981; O'Keefe & Conway, 1978; O'Keefe & Nadel, 1978). Controls and hippocampectomized animals all spent most of their time stopped in front of

Figure 6.5. Mean and standard error of the average speed traveled in each quadrant in the Proximal Probe experiment. A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant quadrant effect ($F(3,30)=3.51$; $p<0.027$).

Proximal Cue Probe: Average Speed

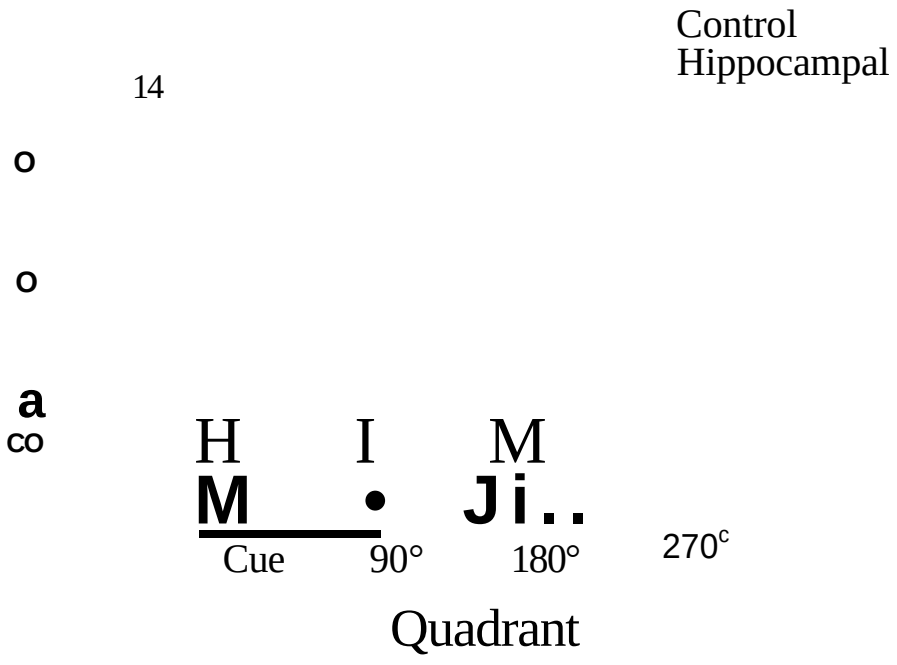


Table 6.3. Post Hoc comparison of the average speed traveled in each quadrant in the Proximal Probe experiment.

		90°	180°	270°
Cue		*	—	*
90°	*	, #f •« 4 n» W * f * *	-	—
180°	—		- S *	
270°	*			r r ,•" : *

1

the cue and continued this behaviour when the cue was removed. Both groups organized their behaviour around the old cue location.

The results of this experiment and the previous experiments suggest that both control and hippocampectomized animals organize their behaviours around proximal cues and that these cues have a flexible representation. This result is consistent with recent findings that hippocampectomized rats can learn places in learning tasks (Whishaw, Cassel, & Jarrad, 1995). The salient nature of the proximal cue might afford information that is easier to learn. The role of learning in home base formation when the proximal cues are not available is examined in the next experiment by using a distal cue task to examine learning and the home base formation.

CHAPTER 7

EXPERIMENT 5 - DISTAL CUE REMOVAL AND SPATIAL LEARNING

/. *Introduction*

The findings of Experiment 4 show a single cue's removal does not disrupt the learning of the home base location. This finding suggested that a constellation of cues is responsible for the formation of the home base. Findings from place cell experiments suggest that the hippocampus is necessary to optimize spatial information and required for flexible representations of the environment. Although hippocampectomized animals were flexible enough to use a proximal cue to learn the constellation of room cues, it is possible that a distal cue is not salient enough to offer this flexibility.

The present experiment asks whether the hippocampus is necessary for home base behaviour when a distal cue is manipulated. Rats were first exposed to a environment with a salient distal cue, and then the cue was removed. In experiment 4 it was found that animals could display spatial learning when trained to a location over 4 days using a proximal cue. The present experiment examined whether rats could form and maintain a home base in response to distal cues after a salient distal cue is removed and what role the hippocampus played in this behaviour.

//. *Methods*

i. Distal Cue Probe

A total of 12 female Long-Evans rats were assigned to a control (n=6) and hippocampal (n=6) groups. In the Distal Cue probe the table that served as an

experimental apparatus was divided into four equal quadrants. The division of these quadrants was such that the book case was positioned at the centre of the cue quadrant. Animals were placed in the centre of the table facing randomized compass positions. Trials were 30 minutes in duration and subjects received one trial per day over a four-day period. Animals were tested for four days with the Dark Sheet in the Cue quadrant and on the fifth day (probe day) the Dark Sheet cue was removed and replaced with a White Sheet. Animals were tested on the probe day with the same protocol as the previous four days.

///. *Results*

i. Time at Cue per Day

There was a significant day by group effect ($F(4,40)=2.90$; $p<0.034$; Fig. 7.1). Subsequent multiple comparison (Bonferroni LSD; $p<0.001$) revealed that the day x group effect was on day 3 and the probe day. The hippocampal group spent more time at the cue location on both days with mean times of 1757.07 seconds (±208.12) and 1790.31 seconds (±115.09) respectively. There were no significant group, day, quadrant x group, day x quadrant, or day x quadrant x group effect for the time spent at the cue (or old cue location) per day. These results show that both groups are flexible in the cues that they use to form the home base location and that hippocampectomized animals spend even more time at the cue locations on certain days.

ii. Paths and Stops

Examination of the paths and stops revealed the location of the home base to be influenced by the old Distal Cue (Fig. 7.2). Animals set up their home base in front of

Figure 7.1. Mean and standard error of the time spent at the cue per day in the Distal Probe experiment. There was a significant effect of day by group ($F(4,40)=2.90$; $p<0.034$).

Distal Cue Probe: Time at cue per day

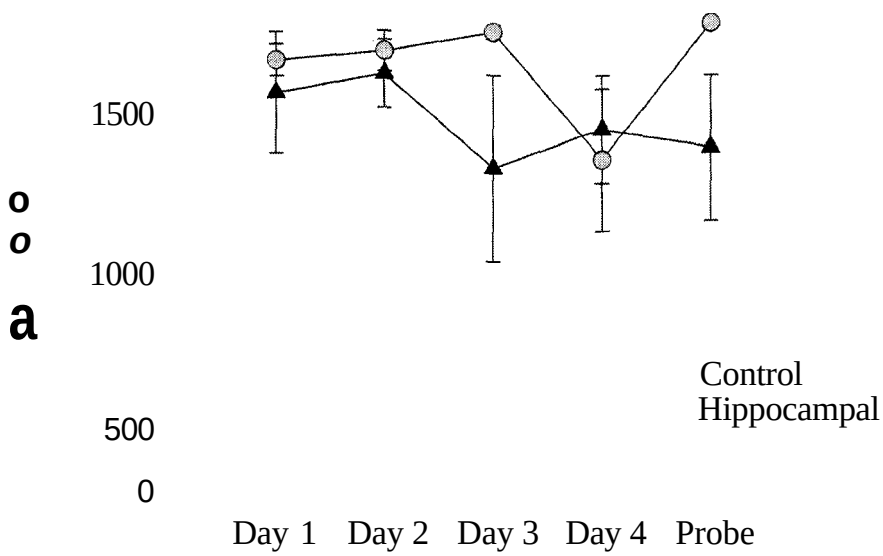
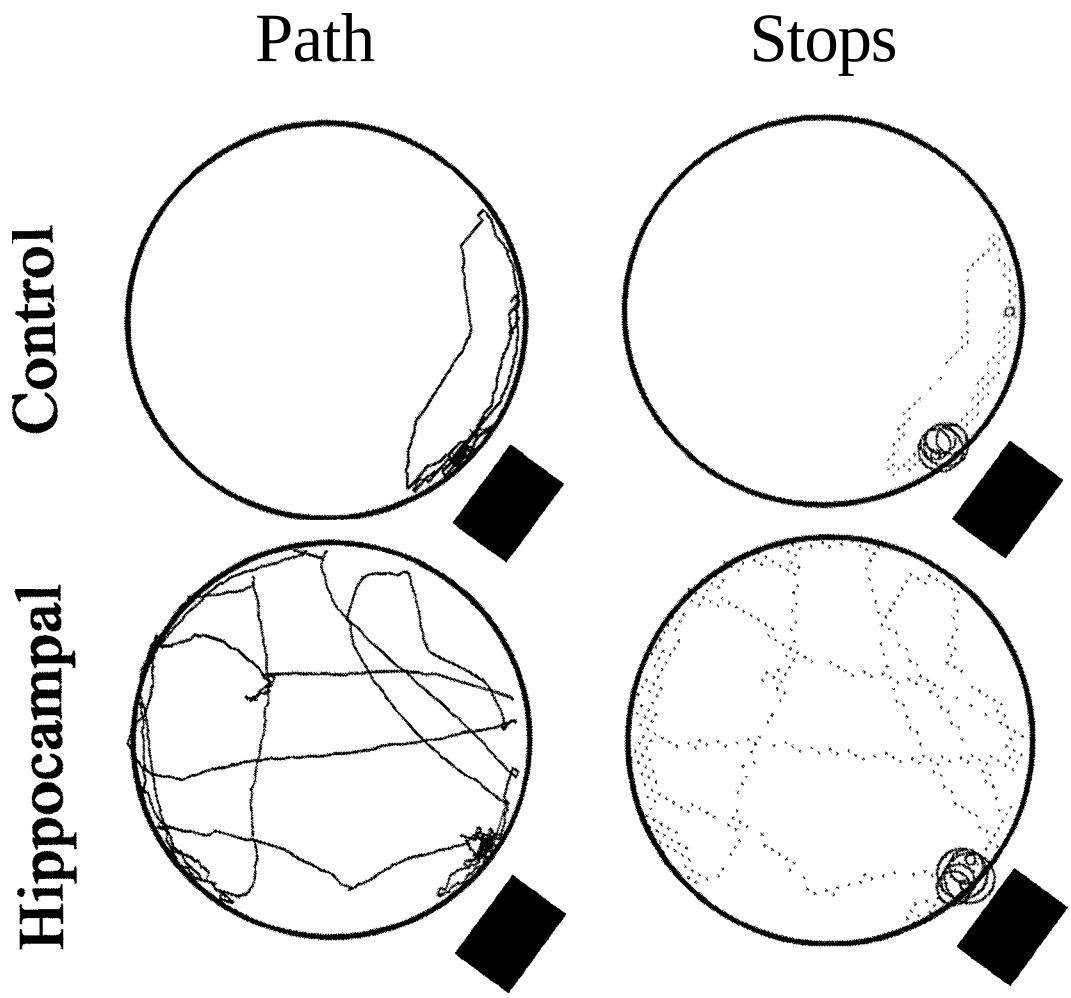


Figure 7.2. Paths and stops for typical control and hippocampectomized rats in the Distal Probe experiment. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. Stops are represented by circles, with diameters corresponding to the amount of time spent at that location. This pattern of behaviour suggests that animals set up their home base in front of the Distal Cue.

Distal Cue Probe



the old Distal Cue, represented by circles with diameters corresponding to the amount of time spent at that location. Typical home base behaviours were also seen at these locations. Path analysis shows that animals make trips to and from the home base. When returning to the home base, animals take trips that are more direct when compared to the trips outward from the home base. The direct nature of paths around the old distal cue further highlights the creation of a home base in front of the old distal cue location during.

iii. Quadrant Time

A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect quadrant ($F(3,30)=79.782$; $p<0.0001$; Fig. 7.3). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparison (Bonferroni LSD; $p<0.0001$; Table 7.1) revealed that quadrant that previously contained the cue before the probe was where animals spent the majority of their time. The quadrant effect was only seen in the Cue quadrant and animals had a mean of total time 1593.50 seconds (±115.09) on the probe day. These results demonstrate that both control and hippocampectomized animals spend most of their time in the old distal cue quadrant. Coupled with an examination of the animals' paths and stops, we further see that animals are stopped in front of the old distal cue location.

Figure 7.3. Mean and standard error of the total time spent in each quadrant. A within subjects analysis of variance on the amount of time spent in each quadrant revealed a significant effect quadrant ($F(3,30)=79.782$; $p<0.0001$).

Distal Cue Probe: Quadrant Time

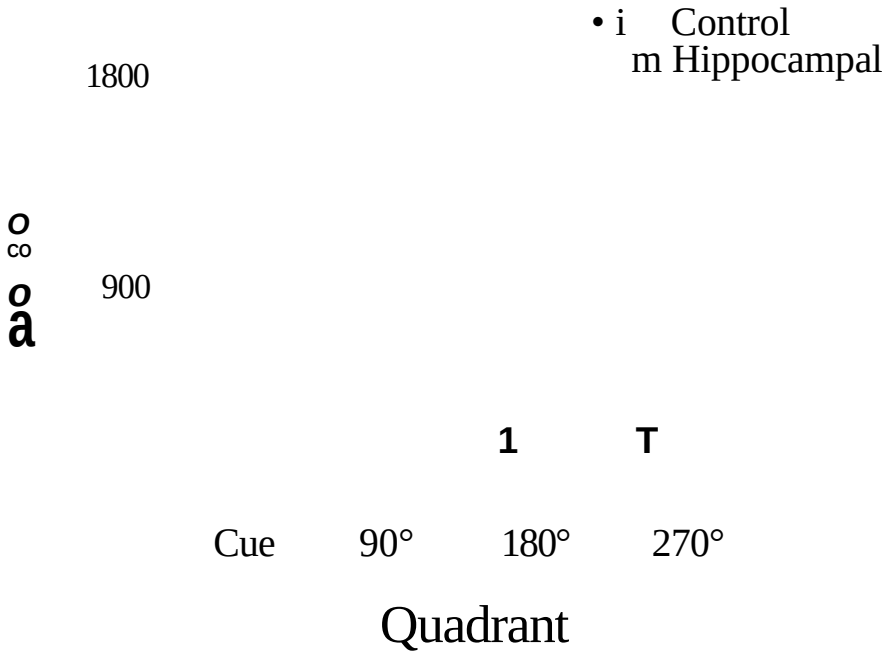


Table 7.1. Post Hoc comparison of the total time spent in each quadrant in the Distal Probe experiment.

in ^ 4	Cue	90°	180°	270°
Cue		***	**#	***
90°	*>i>.	[•		—
180°	***			—
270°	***		—	

iv. Quadrant Distance

A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant effect of quadrant ($F(3,30)=13.373$; $p<0.0001$; Fig. 7.4). There was also a quadrant x group effect on distance ($F(3,30)=4.430$; $p=0.011$). There was no significant effect of group, day, day x group, day x quadrant, or day x group x quadrant. A subsequent multiple comparison (Bonferroni LSD; $p=0.005$; Table 7.2) revealed that the difference in distance was between the old distal cue location and the other quadrants quadrant. Animals had a mean of total distance in the Cue quadrant of 914.37 cm (±290.17) per day. These results also show that both groups, control and hippocampectomized animals, traveled significantly more in the old cue quadrant when compared to the other quadrants. Coupled with the significant amount of time that animals spent in the cue quadrant, we see that animals are largely immobile in the old cue quadrant and have therefore set up a home base

v. Quadrant Speed

A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant quadrant effect ($F(3,30)=3.44$; $p<0.029$; Fig. 7.5). There was no significant group, day, quadrant x group, day x group, day x quadrant, or day x quadrant x group effect. Subsequent multiple comparison (Bonferroni LSD; $p<0.05$; Table 7.3) revealed that the Cue quadrant was where animals traveled at the slowest speeds when compared to the 90° quadrant. Animals had a mean speed in the Cue quadrant of 1.026 cm/second (±0.479) per day. The significantly lower speed in the Cue

Figure 7.4. Mean and standard error of the total distance traveled in each quadrant in the Distal Probe experiment. A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant effect of quadrant ($F(3,30)=13.373$; $p<0.0001$).

Distal Cue Probe: Distance

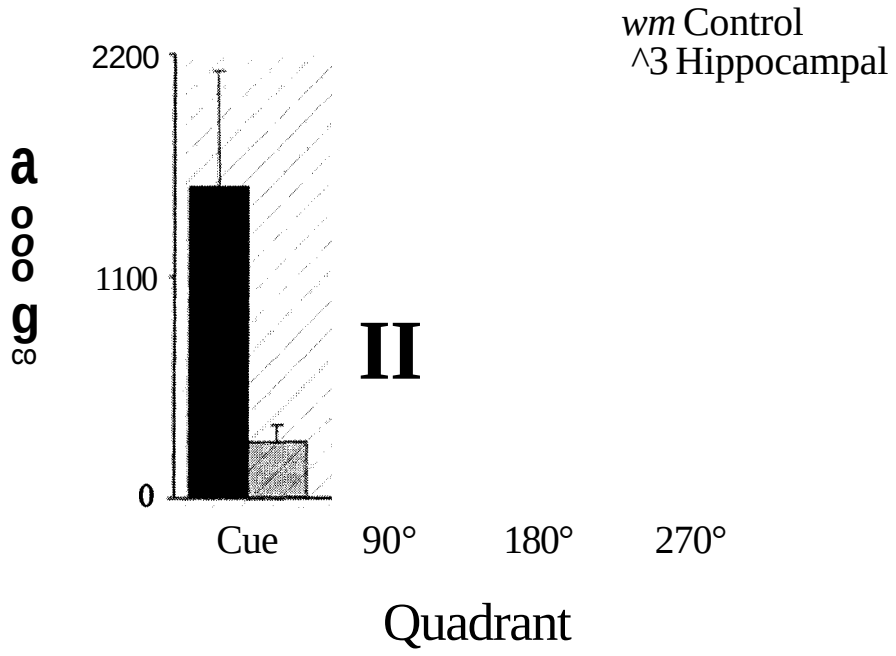


Table 7.2. Post Hoc comparison of the distance traveled in each quadrant in the Distal Probe experiment.

	Cue	90°	180°	270°
Cue		SJCJj»	#*	**
90°	**			—
180°	**			—
270°	**	—		V.».'. y.':'" - is » (Ti"'!""". . . . . ."

Figure 7.5. Mean and standard error of the average speed traveled in each quadrant in the Distal Probe experiment. A within subjects analysis of variance on the amount of distance traveled in each quadrant revealed a significant quadrant effect ($F(3,30)=3.44$; $p<0.029$).

Distal Cue Probe: Average Speed

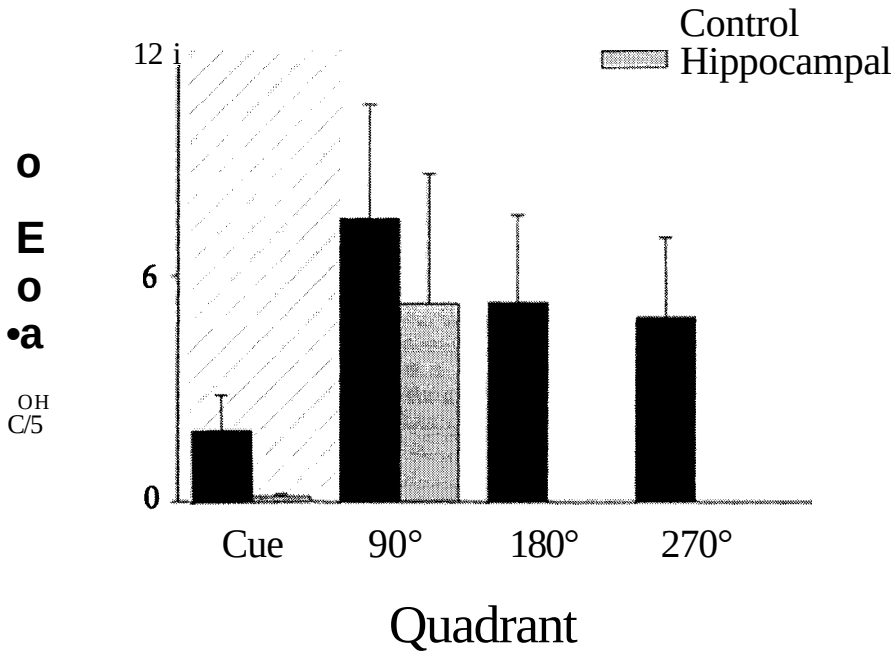


Table 7.3. Post Hoc comparison of the average speed traveled in each quadrant in the Distal Probe experiment.

*' *"J"» — .. .	Cue	90°	180°	270°
Cue	*	*	—	—
90°	*			—
180°	—			—
270°	—	—	—	

quadrant also suggests that animals spend most of their time at low speeds or stopped in front of the old cue location.

IV. Discussion

In this experiment the home base behaviour confirmed the hypothesis that distal cue removal does not create inflexible behavioural strategies in either group. Both control and hippocampectomized animals organized their spatial environment using the distal cue and when the cue was removed both groups exhibited a flexible strategy by both being able to locate the previous location of the home base with no obvious group differences. The present study suggests that hippocampectomized animals are capable of flexible spatial representations.

The present study does result in a striking difference with the literature concerning the role of the hippocampus in spatial learning and organization (Morris, 1981; O'Keefe & Conway, 1978). The difference is created by not finding a group effect on the probe day. Controls and hippocampectomized animals all spent most of their time stopped in front of the cue and continued this trend when the cue was removed. In conjunction with the graphs showing representative paths and stops we find both groups organizing behaviour around the old cue location and therefore the home base. Home base behaviours are one of the most necessary and organized set of behaviours for spatial organization and exhibit a large degree of flexibility in spatial strategy (Eilam & Golani, 1989).

The results of this experiment and the previous experiments suggest that both control and hippocampectomized animals organize their behaviours around distal cues and that these

cues have a flexible representation because animals are not required to use a single cue or representation to navigate effectively. This organization has previously been thought to be hippocampal dependent (O'Keefe and Conway 1978; O'Keefe and Nadel 1978; Morris 1981). It is clear that this representation is flexible but not exclusive to the hippocampus.

CHAPTER 8

GENERAL DISCUSSION

7. *Summary*

The objective of the present thesis was to examine the exploratory behaviour of rats by using their home base behaviour as a focal point and to further examine the role of the hippocampus in spatial navigation. Other researchers have studied exploration behaviours but have not specifically examined the home base behaviour (Whishaw et al., 2001). In a first set of experiments, both control and hippocampectomized rats were found to form a home base using either proximal or distal cues. In a second set of experiments both control and hippocampectomized rats were found to form a home base using a constellation of distal cues. In conditions in this thesis there were no differences between control and hippocampectomized animals in their use of visual cues to set up a home base. Except in conditions requiring self-movement, organized exploration around a home base is not hippocampal dependent

//. *Overview of Experimental Design*

i. Method of Study

This thesis was designed to examine exploratory behaviour using an ethological paradigm. The task used, the open field, offers numerous advantages over other apparatuses used to study spatial behaviours (Eilam and Golani, 1989). Firstly, animals are allowed to behave freely. This point contrasts the water maze where if animals stop

swimming the obvious consequence is drowning (Morris, 1982). The water maze also inhibits an animal's ability to be responsive to changes in body movements and sensory flow that are present on dryland (Whishaw et al., 1995). Secondly, when compared to a radial arm maze, the open field offers animals choice in their direction (Olton et al. 1979). Therefore, the ability stop and start and move in any direction offers an ethological approach to studying spatial behaviour and allows for a broader examination of spatial choices made by rats.

The shape and size of the table also acted to promote the use of cues in the environment. A circular table provides advantages by not allowing reference points that would be created at the corners of a typical maze. Any apparatus with more than two corners creates geometric cues that can work in relation with each other to help animals navigate. The large size of the table also helps to disambiguate spatial behaviour by not constraining the length of the trips that rats may take. The table was also mounted on a ball bearing platform and could be rotated in any direction. An effective strategy for minimized olfactory cue use was to clean and rotate the table between rats.

Rats were also given a longer period of time to perform the task when compared to traditional navigational tasks. Further, animals were not stressed by food or water deprivation and were given ad libitum access to the aforementioned. All these conditions served to create a task that promoted as natural exploration as possible in the laboratory.

The animals were video recorded from a camera directly above and perpendicular to the table surface. The camera also was equipped with an infrared lens so that rats movements could be tracked in total darkness. The location of the camera reduced the lensing effects that are created when objects are viewed at an angle. This reduction was

necessary to obtain constant measurement in all portions of the table. From these recordings, sets of x,y coordinates were produced that represented the animals' movements in space. The set of x,y coordinates were transformed into speeds and distances travelled by the rats during the testing session.

One measure that was derived from the speed data was stopping. Stopping was defined as the cessation of movement for a period of greater than one second. Stopping behaviour was located spatially by filtering x,y data points into segments that met the criteria for stopping and then plotting these locations. The home base was defined in a similar manner, with the basic definition for a home base being the location where animals stopped for the longest cumulative time.

ii. Cues

The types of cues that were used, proximal and distal, were chosen because of their contrasting relevance in spatial navigation. Proximal cues tend to dominate the visual field and therefore act as beacon for navigation. Distal cues are in constellations because they offer information about the relationship of an animal to its world. Both proximal and distal cues used in this experiment were common items found in research laboratories. The effect that these cues have on spatial behaviours suggest that researchers should be aware of the design and layout of their testing facilities and also the salience of the cues that they use.

The results of the experiments also suggest that the physical properties of the individual cue affects the saliency of the cue. Prusky et al., (2000) found that visual acuity and the apparent salience of a cue affect an animal's ability to form a place

response. Visual salience can be described as how easily a cue can be distinguished compared to other cues. Thus distinguishing features could be related to both the colour gradient, three-dimensional nature of a cue, and the amount of visual field that the cue occupies (Prusky et al., 2000). Placing the dark sheet on the bookcase caused animals to use the cue for home base formation while placing a white sheet did not. The proximal cue was a black three-dimensional object providing a high degree of visual salience and influenced the location of the home base.

When the relatively low visual acuity of rats is considered, about 1 cycle per degree, the overall size, contrast and illumination of the cues should plays a main role in an animals ability to perform tasks that require effectively using visual cues (Prusky, 2000).

Although a cage or cue could have been placed directly on the table, a decision was made to use extra-maze cues. This decision was important because intra-maze cues offer tactile cue information and the thesis was mainly concerned with visual cue use. Tactile cues from a home cage would provide a constant source of information about the home base that could be used for taxon strategies. The experiments would therefore have been constrained to only examining taxon strategies.

III. Navigational Strategies

i. Taxon

The results of the first two experiments confirm the hypothesis that rats can use taxon cues to form a home base and that this behaviour is not hippocampal dependent. Taxon cues provide information that allows and animal to move towards or away from

that cue and acts as a beacon to guide behaviour. In the first experiment both control and hippocampectomized rats formed home bases using the large black box that served as a proximal cue. In the second experiment animals used a distal cue that was a large book case with and without a dark or white sheet placed over it. Animals used the distal cue to form a home base with the book case or dark sheet cues. With the white sheet was placed over the home base, the visual saliency of the cue was decreased and animals used other cues in the environment to form the home base. These results further suggest that control and hippocampectomized rats both use strategies that require visual cues to form the home base. Therefore, although the home base has been previously stated as the first behaviour in a series of behaviours designed to organize the environment, cue use and interpretation might be the first step in organizing an environment. Rats in all experiments chose a specific cue and then initiated the ordering of behaviours around the cue. The formation of home bases by hippocampectomized animals also suggests that this initial ordering of the spatial behaviours is not reliant on the hippocampus.

ii. Locale

The repertoire of home base behaviours is one of the most necessary and organized sets of spatial behaviours. Locale navigation uses a group or ensemble of cues in the environment to create a map and then calculate a path.

The results the first two experiments suggest that both control and hippocampectomized animals organize their behaviours around salient distal and proximal cues. The fourth experiment removed the primary proximal cue after four days of training and found that control or hippocampectomized animals' spatial memory for

the home base location was not affected. The fifth experiment removed the distal cue after four days of training and found control or hippocampectomized rats were capable of using the constellation of cues in the room to remain at the home base. In both the fourth and fifth experiments involving cue removal, rats did not disregard the old home base location and form a new home base in relation to a new taxic cue. This suggests that both control and hippocampectomized animals organize their behaviours using a flexible representation and did not rely on a solitary cue.

Although the experiment where the proximal cue was removed after four days of pre-exposure found that both controls and hippocampectomized rats learned the location of the home base, it is possible that the other distal cues are capable of polarizing the home base location. From the results of the distal probe experiment, taken in conjunction with the proximal probe experiment, it is clear that the primary cue is not the sole determining factor in home base formation. Both control and hippocampectomized rats appear to use a constellation of cues to form the home base. These results are consistent with previous work showing that hippocampectomized animals are capable of forming a place response (Whishaw, 1985; Gaffan, 1994, Day et a1, 1999).

iii. Self-Movement and Olfactory Cues

Although rats use visual cues, this does not rule out the possibility that self-movement cues are also used for home base formation. The results from the total darkness experiment suggest that self-movement cues are important for navigating in total darkness in control animals. Hippocampectomized animals did not form a home base in the dark despite the availability of olfactory cues. This suggests that olfactory

cues do not play a critical role in home base formation, however self-movement cues may be essential. The ability of controls to detect urine or other odours emitted as soon as the animal enters the apparatus, along with dead reckoning, would allow an animal to immediately and continually return to the same position (Whishaw et al., 1997; Whishaw &Tomie, 1997).

An examination reveals that the dispersion of stops is much higher in front of the home base in the dark for control and bulbectomized rats. This further suggests that in the dark, control and bulbectomized animals use the process of dead reckoning in the absence of visual cues. The error created by dead reckoning could possibly be factored out by incorporating weaker and less salient odour cues. Although bulbectomized animals also have access to self-movement cues, large amounts of error would initially be built up and it would take time to recalibrate their environment using these cues. This is a further possibility for why bulbectomized animals form random home bases where as control home base formation is largely confined to the 270° quadrant. Regardless of this speculation, it is clear that hippocampetomized animals are not capable of creating home bases in total darkness where as controls and bulbectomized animals are.

These results from the first set of three experiments confirms that the home base is central to the exploratory behaviour of animals and that home base formation is influenced by cues in both control and hippocampectomized animals.

iv. The Home Base and the Hippocampus

In all experiments, a group of animals received bilateral hippocampectomies and served to investigate the role of the hippocampus in home base behaviour. No difference

between control and hippocampectmized rats in was found in the measures of time spent, distance travelled, and speed in the quadrants containing the proximal and distal cues. An examination of regions where rats stopped found them stopping most in front of a salient proximal and distal cue and this behaviour was not hippocampal dependant.

There were also no differences between control and hippocampectomized animals in their ability to form home bases when cues were removed. Further, these experiments suggested that control and hippocampectomized animals use cues other than the primary salient cue to form the home base. This flexible representation relies on an association between the primary cue and other distal cues in the environment.

A major difference between control and hippocampectomized groups was found when all visual cues where removed. During the total darkness manipulation, hippocampectomized rats were not capable of forming a home base. Control and olfactory bulbectomized animals both formed home bases suggesting that visual and olfactory cues are not required for this behaviour. It is possible those self-movement cues play a role in home base formation and that the hippocampus is a facilitating structure for self movement strategies. This finding is consistent with other research showing that dead reckoning is important for organized navigation (Wallace et al., 2001).

IV. Interpretations

i. Spatial Mapping Theory

One of the major theories of hippocampal function is O'Keefe and Nadel's (1978) spatial mapping theory. A major tenant of spatial mapping theory is that the hippocampus is important for exploratory behaviour and provides a representation of the

world. When O'Keefe and Nadel (1978) talk about what exploration will look like in animals with hippocampal lesions they are not specific. However, they do claim that hippocampectomised animals should not have any organized behaviour. With respect to home base behaviour, the present thesis does not support this prediction because both control and hippocampectomized animals formed home bases using proximal, distal, and a constellation of cues. Hippocampectomized animals not only displayed organized behaviour, but also can use both a taxon and a locale strategy to do so.

ii. Configural Theory

Another theory that attempts to make predictions about hippocampal function and memory is the configural Learning Theory (Rudy and Sutherland, 1989). Rudy and Sutherland suggest that all learning fits into to elemental and configural categories. Elemental learning involves one cue and is not complex, where "a configural approach involves a cue in association with one or more cues. Configural learning is more complex than elemental learning and requires the hippocampus. A key difference between spatial mapping theory and configural theory is that the former is purely topographical in nature. Evidence from the present thesis suggests that both control and hippocampectomized animals were capable of using a configuration of cues to create a home base. Configural theory predicts that hippocampectomized animals cannot establish a home base using proximal, distal and a configuration of cues. Specifically, rats in the experiments where cues where removed showed learning of a configural problem.

iii. Piloting and Dead Reckoning

Another theory of how animals solve spatial problems comes from Gallistel (1990). Gallistel's theory on navigation involves a record in the central nervous system of geometric relations among surfaces. This record is used to plan movements through the environment. The information that is encoded by the hippocampus in this process is the relation between geometric values. Animals use these pieces of geometrical information to create a cognitive map in the brain.

Two strategies that animals use while navigating are piloting and dead reckoning. Piloting involves the use of one or more visual cues to reach a goal. The complexity of the piloting strategies varies depending on the number of cues that are used to pilot. More complex strategies incorporate the relationship between many cues while simpler strategies involve a single cue.

Dead reckoning is a strategy whereby an animal monitors all of its movements and uses this record to calculate the distance and direction from its starting point. Although dead reckoning can be used as a method of navigation alone, large amounts of error accumulate. Dead Reckoning can be used in conjunction with visual cues to correct these errors.

The current thesis' findings suggest that both control and hippocampectomized animals can use visual cues and therefore pilot effectively. When animals are tested in the dark, thus removing visible cues, only control animals can effectively dead reckon. These results are consistent with studies examining homing behaviours in rats (Whishaw 1998; Whishaw etal., 2001).

iv. Hippocampus and Movement

Another theory is that the hippocampus does not have a single function but is involved in a number of subsystems of behaviour (Bland,1986). One of the well defined physiological properties of the hippocampus is the rhythmical slow activity called theta. Theta is a 3-12 hertz oscillation that serves to synchronize voluntary motor movements in animals (Bland and Colom, 1993). Whishaw and Vanderwolf (1973) found that the amplitude of the theta rhythms recorded in moving animals increased in relation to amplitude of the movement that they had executed. For instance, when theta was recorded in animals that had been taught to jump to avoid a foot shock, theta rythms amplitude increased as the height of the jump also increased. These results suggest that the hippocampus is involved in integrating sensory information and then selects the appropriate voluntary motor response (Oddie and Bland, 1998). The current thesis' findings suggest that both control and hippocampectomized animals can use visual cues and navigate effectively. However, when animals are tested in the dark, thus removing visible cues, only control animals can effectively execute the appropriate behaviours using self movement. These results are consistent with Blands (1986) theory of hippocampal involvement in movement control.

V. Relevance

Prior to this thesis, various researchers have reported that cells in the hippocampus selectively respond to cues. A question that arises from these studies is

"What do these cues signal?" The present thesis found that select cues play a major role in the formation of the home base. It is also possible that the hippocampal place cells are encoding something else and are not necessary for place representation.

Further research has also shown that hippocampal place cells are involved in dead reckoning (McNaughton et al., 1993, Knierim 1994, Taube 1993). Results from the thesis suggest that the hippocampus is not exclusively involved in place representation but is involved in dead reckoning.

The conclusion that can be drawn from the thesis is that most researchers do not consider how organized rat behaviour actually is. An interesting study would be to examine place cells and the home base behaviour congruently.

VI. General Conclusion

The present thesis used home base behaviour to examine one facet of exploration using an ethological method. Control and hippocampectomized rats used both taxon and locale strategies and showed configural learning. Hipocampal animals where impaired on tasks where visual cue were not present and had problems consistent with an impaired dead reckoning strategies.

Previous research (Wallace et al., 2001) has shown that the inward portion of exploration is organized around visual cues and that in the absence of these cue only hippocampectomized animals become disorganized. The present thesis also showed that the home base portion of exploration involved visual cue use and also became disorganized in hippocampectomized rats when visual cues were not present. The outward trip portion of exploratory behaviour is still to be examined but these findings

would predict that hippocampectomized animals would appear normal until visual cues were absent and they were forced to use self-movement to guide their behaviour.

REFERENCES

Aguirre, G. K., J. A. Detre, et al. (1996). "The parahippocampus subserves topographical learning in man." Cereb Cortex 6(6): 823-9.

Amaral, D. G. and M. P. Witter (1989). "The three-dimensional organization of the hippocampal formation: a review of anatomical data." Neuroscience 31(3): 571-91.

Barnea, A. and F. Nottebohm (1994). "Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees." Proc Natl Acad Sci U S A 91(23): 11217-21.

Basil, J. A. (1993). Neuroanatomical and Behavioral Correlates of Spatial Memory in Clark's Nutcrackers., University of Massachusetts: 221.

Best, P. J. and A. M. White (1998). "Hippocampal cellular activity: a brief history of space." Proc Natl Acad Sci U S A 95(6): 2717-9.

Best, P. J. and A. M. White (1999). "Placing hippocampal single-unit studies in a historical context." Hippocampus 9(4): 346-51.

Best, P. J., A. M. White, et al. (2001). "Spatial processing in the brain: the activity of hippocampal place cells." Annu Rev Neurosci 24: 459-86.

Blackstad, T. W. (1969). "Studies on the hippocampus: methods of analysis." UCLA Forum Med Sci 11: 391-414.

Bliss, T. V. and T. Lomo (1973). "Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path." J Phvsiol 232(2): 331-56.

Brodbeck, D. R. and S. J. Shettleworth (1995). "Matching location and color of a compound stimulus: Comparison of a food-storing and a nonstoring bird species." Journal of Experimental Psychology: Animal Behavior Processes 21: 64-77.

Cajal, C. J. (1911). "Histologie du systeme nerveux de l'homme et des vertebres." 2.

DeRenzi, E., Faglioni, P., Villa, P. (1977). "Topographical amnesia." J Neurol Neurosurg Psychiatry 40: 498-505.

Eilam, D. and I. Golani (1989). "Home base behavior of rats (Rattus norvegicus) exploring a novel environment." Behav Brain Res 34(3): 199-211.

Fox, S. E. and J. B. Ranck, Jr. (1975). "Localization and anatomical identification of theta and complex spike cells in dorsal hippocampal formation of rats." Exp Neurol 49(1 Ptl): 299-313.

Hagan, J. J., J. D. Salamone, et al. (1988). "Place navigation in rats is impaired by lesions of medial septum and diagonal band but not nucleus basalis magnocellularis." Behav Brain Res 27(1): 9-20.

Hawkings, S. and W. Penrose (1995). The Nature of Space and Time. Princeton, Princeton University Press.

Hebb, D. O. (1961). The organization of behavior, a neuropsychological theory. New York, Science Edition Inc.

Hetherington, P. A. and M. L. Shapiro (1997). "Hippocampal place fields are altered by the removal of single visual cues in a distance-dependent manner." Behav Neurosci 111(1): 20-34.

Hill, A. J. and P. J. Best (1981). "Effects of deafness and blindness on the spatial correlates of hippocampal unit activity in the rat." Exp Neurol 74(1): 204-17.

Kamil, A. C, R. P. Balda, et al. (1999). "Patterns of movement and orientation during caching and recovery by Clark's nutcrackers, Nucifraga columbiana." Anim Behav 57(6): 1327-1335.

Kelso, S. R., A. H. Ganong, et al. (1986). "Hebbian synapses in hippocampus." Proc Natl AcadSciUSA83(14): 5326-30.

Krebs, J. R., D. F. Sherry, et al. (1989). "Hippocampal specialization of food-storing birds." Proc Natl Acad Sci U S A 86(4): 1388-92.

Krushinskaya, N. L. (1966). "Some complex forms of feeding behaviour of nutcracker Nucifraga caryocatactes, after removal of old cortex." Journal of evolutionary biochemistry and physiology. II: 563-568.

Kuhn, T., S. (1970). The Structure of Scientific Revolutions. Chicago, The University of Chicago Press.

Lorente De No, R. (1933). "Studies on the structure of the cerebral cortex. I. The area entorhinalis." Journal fur Psychologie und Neurologie 45: 381-438.

Maguire, E. A., T. Burke, et al. (1996). "Topographical disorientation following unilateral temporal lobe lesions in humans." Neuropsychologia 34(10): 993-1001.

Maguire, E. A., R. S. Frackowiak, et al. (1997). "Recalling routes around london: activation of the right hippocampus in taxi drivers." J Neurosci 17(18): 7103-10.

McCrone, J. (2003). "Quantum mind." Lancet Neurol 2(7): 450.

Mishkin, M. (1978). "Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus." Nature 273(5660): 297-8.

Morris, R. G., P. Garrud, et al. (1982). "Place navigation impaired in rats with hippocampal lesions." Nature 297(5868): 681-3.

Morris, R. G., F. Schenk, et al. (1990). "Ibotenate Lesions of Hippocampus and/or Subiculum: Dissociating Components of Allocentric Spatial Learning." Eur J Neurosci 2(12): 1016-1028.

Morris, R. G. M. (1981). "Spatial localization does not require the presence of local cues." Learning and Motivation 12: 239-261.

Muller, R. U. and J. L. Kubie (1987). "The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells." J Neurosci 7(7): 1951-68.

Neitz, J. and G. H. Jacobs (1986). "Reexamination of spectral mechanisms in the rat (Rattus norvegicus)." J Comp Psychol 100(1): 21-9.

O'Keefe, J. (1976). "Place units in the hippocampus of the freely moving rat." Exp Neurol 51(1): 78-109.

O'Keefe, J. and D. H. Conway (1978). "Hippocampal place units in the freely moving rat: why they fire where they fire." Exp Brain Res 31(4): 573-90.

O'Keefe, J. and J. Dostrovsky (1971). "The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat." Brain Res 34(1): 171-5.

O'Keefe, J. M. and L. Nadel (1978). The hippocampus as a cognitive map. Oxford New York, Clarendon Press ;
Oxford University Press.

Olton, D. S. (1983). Memory functions and the hippocampus. Neurobiology of the hippocampus. W. Seifert. New York, Academic Press: 335-374.

Olton, D. S. and B. C. Papas (1979). "Spatial memory and hippocampal function." Neuropsychologia 17.

Olton, D. S. and R. J. Samuelson (1976). "Remembrance of places passed: spatial memory in rats." Journal of Experimental Psychology: Animal Behavior Processes 2: 97-116.

Quirk, G. J., R. U. Muller, et al. (1990). "The firing of hippocampal place cells in the dark depends on the rat's recent experience." J Neurosci 10(6): 2008-17.

Ranck, J. B., Jr. (1973). "Studies on single neurons in dorsal hippocampal formation and septum in unrestrained rats. I. Behavioral correlates and firing repertoires." Exp Neurol 41(2): 461-531.

Restle, F. (1957). "Discrimination of Cues in Mazes: A resolution of the "place-vs-response" question." Psychological Review 64: 217-28.

Sachs, M. (1996). "Changes in Concepts of Time from Aristotle to Einstein." Astrophysics and Space Science 244: 269.

Shapiro, M. L., H. Tanila, et al. (1997). "Cues that hippocampal place cells encode: dynamic and hierarchical representation of local and distal stimuli." Hippocampus 7(6): 624-42.

Sherry, D. and S. Duff (1996). "Behavioural and neural bases of orientation in food-storing birds." J Exp Biol 199(Pt 1): 165-72.

Shillito, E. (1963). "Exploratory Behaviour in the Short-tailed Vole Microtus Agrestis." Behaviour 21: 145-154.

Skelton, R. W. and R. K. McNamara (1992). "Bilateral knife cuts to the perforant path disrupt spatial learning in the Morris water maze." Hippocampus 2(1): 73-80.

Smulders, T. V., A. D. Sasson, et al. (1995). "Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee." J Neurobiol 27(1): 15-25.

Sutherland, R. J. and R. H. Dyck (1982). "Place navigation by rats in a swimming pool." Canadian Journal of Psychology 38: 322-347.

Sutherland, R. J., B. Kolb, et al. (1982). "Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat." Neurosci Lett 31(3): 271-6.

Sutherland, R. J., M. P. Weisend, et al. (2001). "Retrograde amnesia after hippocampal damage: recent vs. remote memories in two tasks." Hippocampus 11(1): 27-42.

Sutherland, R. J., I. Q. Whishaw, et al. (1983). "A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat." Behav Brain Res 7(2): 133-53.

Swanson, L. W. and W. M. Cowan (1977). "An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat." J Comp Neurol 172(1): 49-84.

Taube, J. S. (1995). "Head direction cells recorded in the anterior thalamic nuclei of freely moving rats." J Neurosci 15(1 Pt 1): 70-86.

Taube, J. S. (1998). "Head direction cells and the neurophysiological basis for a sense of direction." Prog Neurobiol 55(3): 225-56.

Thompson, L. T. and P. J. Best (1989). "Place cells and silent cells in the hippocampus of freely-behaving rats." J Neurosci 9(7): 2382-90.

Tolman, E. C. (1948). "Cognitive maps in rats and men." The Psychological Review 55(4): 189-208.

Tomback, D. F. (1980). "How nutcrackers find their seed stores." Condor 82: 10-19.

Vander Wall, S. B. (1990). Food Hoarding in Animals. Chicago, University of Chicago Press.

Wallace, D. G., B. Gorny, et al. (2002). "Rats can track odors, other rats, and themselves: implications for the study of spatial behavior." Behav Brain Res 131(1-2): 185-92.

Whishaw, I. Q. (1985). "Formation of a place learning-set by the rat: a new paradigm for neurobehavioral studies." Physiol Behav 35(1): 139-43.

Whishaw, I. Q., J. C. Cassel, et al. (1995). "Rats with fimbria-fornix lesions display a place response in a swimming pool: a dissociation between getting there and knowing where." J Neurosci 15(8): 5779-88.

Whishaw, I. Q., D. J. Hines, et al. (2001). "Dead reckoning (path integration) requires the hippocampal formation: evidence from spontaneous exploration and spatial learning tasks in light (allothetic) and dark (idiothetic) tests." Behav Brain Res 127(1-2): 49-69.

Whishaw, I. Q., J. E. McKenna, et al. (1997). "Hippocampal lesions and path integration." Curr Opin Neurobiol 7(2): 228-34.

Whishaw, I. Q. and J. A. Tomie (1997). "Piloting and dead reckoning dissociated by fimbria-fornix lesions in a rat food carrying task." Behav Brain Res 89(1-2): 87-97.

Zola-Morgan, S. and L. R. Squire (1985). "Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia." Behav Neurosci 99(1): 22-34.

APPENDIX A

C++ Programs

7. C++ *Program written for Stops*

```
#include <stdlib.h>
#include <stdio.h>
#include <math.h>
#include <time.h>
#include "allegro.h"

#define SIZE 80

int the_data[SIZE][SIZE];
int i;
 BITMAP* buffer;
        FILE *fp;
        charbuf[80];
        char var_buf_l[80], var_buf_2[80];
        float x, y;
        intnx, ny, j;
        int the_max = 0;
```

```
/hi* h^ ht' h^ ht' h^ hV h^ h^ hi* hi* ht* hi* hhf hi* hi' hi* ht* ht' hi* ht' hi« hf* h!' hi* hi' hi' hi,* h^ h^ hi* h^ h^ hi* h^ h^ hi* h^ hi* hi* h^ hi* h^ ht' hi* h^ hi' hi* hi' hi' *I* hi* ^* h^ ^U h^ ^U h^ h^ h^ h|* h^ h^ h^ hl* h^ hi' ^* h^ h^ hi*
/ ^^ ^^ ^^ ^^ ^^ ^^ *n #^ ^^ ^p ^^ «^ ^p ?|?> *p ?S ^p r p rj> *p ?|?> #I> *|> *|> ^S ^^ ^^ ^* ^p *p ^% ^^ ^^ ^> *^ rp ^p *^ ^^ ^^ *^ ^^ ^|> ^^ ^^ ^> *p *^ ^^ ^^ ^^ ^^ ^^ *^ ^^ ^p *p ^^ ^^ *p *^ *p *p *^ *p *^ ^p *|* *|* r p *^* *p
```

```
char *get_line(char *string, int maxLength, FILE *fp)
{
char buffer[512]; /* temporary string storage */

int length,      /* length of line read */
   index=0,     /* looping variables */
   index_2=0,
   parsed=0;    /* has the current input line been parsed */

/* get the next line of input, make sure there is something on the line */

while(l)
    {
    if   (!fgets(buffer,maxLength,fp))
          return (NULL);
    length = strlen(buffer);

    /* kill the carriage return */
```

```
buffer[length-l] = 0;

/* reset index */

index = 0;

/* eat leading white space */

while(buffer[index]=-  ')
        index++;

/* read line into buffer, if "#" arrives in data stream then disregard
 rest of line */

parsed=0;
index_2=0;

while ([parsed)
        {

        if  (buffer[index] !='#')
          {
          /* insert character into output string */

          string [index_2] = buff er [index];

          /* test if this is a null terminator */

          if  (string[index_2]==0)
                parsed=l;

          /* move to next character */

          index++;
          index_2++;

          }
        else
          {
          /* insert a null termination since this is the end of the string */
          string [index_2] = 0;
          parsed=l;
          }
        }
        /* make sure there's a string there and not a blank line */
```

```
            if (strlen(string))
              return (string);

    }
}


int main(int argc, char **argv[])
{

  FILE *fp;
  PALETTE p;
  RGB temp;
  BITMAP *bmp;
  PALETTE tpal;
  PALETTE pal;
  int intense;
  float the_time;

  charbuf[80];


  allegro_init();
  install_keyboard();
  install_timer();
  install_mouse();




  set_color_depth(16);
  set_gfx_mode(GFX_AUTODETECT_FULLSCREEN, 800, 600, 0, 0);
  buffer = create_bitmap(400, 400);
  //set_palette(desktop_palette);
  generate_332_palette(p);
  /*
    for(i = 0; i < 256; i++)
  {
          p[i].r = p[i].g = p[i].b = i;
  }
  */
   set_palette(p);

  clear_to_color(buffer,  makecol(255,255,255));
  clear_to_color(screen,  makecol(255,255,255));
```

```
//read_data();
        for(i = 0; i < SIZE; i++)
          for(j = 0; j < SIZE; j++)
                    the_data[i][j] = 0;

      sprintf(buf, "%s.txt", argv[l]);
      fp = fopen(buf, "r");
        while(getjine(buf, 80, fp))
        {
                sscanf(buf, "%s %s", var_buf_l, var_buf_2);
                x = atof(var_buf_l);
                y = atof(var_buf_2);
                nx = (int)x/(int)(400/SIZE);
                ny=(int)y/(int)(400/SIZE);
                the_data[nx] [ny]++;
                if (the_data[nx] [ny] > the_max) the_max = the_data[nx] [ny];
        }

        fclose(fp);

        fp = fopenCoutput.txt", "w");
        for(i = 0; i < SIZE; i++)
        {
          for(j = 0; j < SIZE; j++)
           {

            intense = (int)(255 - (255 * (float) ((float)the_data[i]0]/(float)the_max)));
                if (intense < 0) intense = 0;
                fprintf(fp, "%d ", intense);
        /* rectf ill (buffer, i * (int) (400/SIZE),
                        j * (int)(400/SIZE),
                        i * (int)(400/SIZE) + (int)(400/SIZE),
                        j * (int)(400/SIZE) + (int)(400/SIZE),
                        makecol(intense, intense, intense));
        */
                the_time = (float)the_data[i][j]/60;
                if((the_time > 0) && (the_time < 10))
                        circle(buffer,
                        i * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                        j * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                        0,
                        makecol(0,0,0));
                if ((the_time >= 10) && (the_time < 30))
                        circle (buffer,
                        i * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                        j * (int) (400/SIZE) + (int) (400/SIZE)*.5,
```

```
                    3,
                    makecol(0,0,0));
                if((the_time >= 30) && (the_time < 120))
                    circle (buffer,
                    i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                    j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                    10,
                    makecol(0,0,0));
                if((the_time >= 120) && (the_time < 300))
                    circle (buffer,
                    i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                    j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                    15,
                    makecol(0,0,0));
                if (the_time >= 300)
                    circle (buffer,
                    i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                    j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                    20,
                    makecol (0,0,0));

            }
            fprintf(fp, "\n");
          }
          fprintf(fp, "%d", the_max);
          fclose(fp);
          //circleflbuffer, 235,135,130, makecol(0,0,0));
 get_palette(pal);
 sprintf(buf, "%s.bmp", argv[l]);
 save_bitmap(buf, buffer, pal);
  blit(buffer, screen, 0, 0, 0, 0, buffer->w, buffer->h);
  textout_centre(screen, font, "Test", 600, 500, 0);
  //get_palette (tpal);
  //bmp = create_sub_bitmap(screen, 0, 0, SCREEN_W, SCREEN_H);

// while(! (key[KEY_ESC])) {};
 //get_palette(pal);
 //save_bmp("test.bmp", buffer, pal);


  allegro_exit();

  return 0;
}

END_OF_MAIN();
```

//. C++ Program written for Paths

```
#include <stdlib.h>
#include <stdio.h>
#include <math.h>
#include <time.h>

#include "allegro.h"

#define SIZE 80

int the_data[SIZE] [SIZE];
int i;
  BITMAP* buffer;
          FILE *fp;
          charbuf[80];
          char var_buf_l[80], var_buf_2[80];
          float x, y;
          intnx, ny, j;
          int nxx.nyy;
          int the_max = 0;

/ 5|^ r p 5js ?j? 5|* 5|5 5|% ?J? r p ?|* ?|5 ?J* 5J^ ?J5 ?|^ ?J5 ?j^ 5j5 r|^ rj* ?J^ ?J* rj* ?|* ?j^ 5J5 ?JC PJ5 5|^ ?|> ^^ ^C P|* 5|> ?p 5p 5^ ^C ?^ ^C ?^ ^C 5^ 5^ ?^ ?^ 5^ 5^ 5^ ^? 5^ 5^ ?^ 5^ ?^ ?E> ?^ ?^ 5^ ?|5 ?j5 ^5 ?|> ?{5 ?E> ^C ?J5 ^* ?jC ^% 5j€

char *get_line(char *string, int maxLength, FILE *fp)
{
char buffer[512]; /* temporary string storage */

int length,      /* length of line read */
   index=0,     /* looping variables */
   index_2=0,
   parsed=0;    /* has the current input line been parsed */

/* get the next line of input, make sure there is something on the line */

while(l)
    {
    if   (!fgets(buffer,maxLength,fp))
          return (NULL);
    length = strlen(buffer);

    /* kill the carriage return */
```

```
buffer[length-l] = 0;

/* reset index */

index = 0;

/* eat leading white space */

while(buffer[index]=-  ')
        index++;

/* read line into buffer, if "#" arrives in data stream then disregard
 rest of line */

parsed=0;
index_2=0;

while (! parsed)
        {

        if  (buffer[index] !='#')
          {
          /* insert character into output string */

          string[index_2] = buffer [index];

          /* test if this is a null terminator */

          if (string [index_2]==0)
                parsed=l;

          /* move to next character */

          index++;
          index_2++;

          }
        else
          {
          /* insert a null termination since this is the end of the string */
          string [index_2] = 0;
          parsed=l;
          }
        }
        /* make sure there's a string there and not a blank line */
        if  (strlen(string))
```

```
            return (string);

    }
}


int main(int argc, char **argv[])
{

  FILE *fp;
  PALETTE p;
  RGB temp;
  BITMAP *bmp;
  PALETTE tpal;
  PALETTE pal;
  int intense;
  float the_time;

  charbuf[80];


  allegro_init();
  install_keyboard ();
  install_timer();
  install_mouse();




  set_color_depth (16);
  set_gfx_mode(GFX_AUTODETECT_FULLSCREEN, 800, 600, 0, 0);
  buffer = create_bitmap(400, 400);
  //set_palette (desktop_palette);
  generate_332_palette (p);
  /*
    for(i = 0; i < 256; i++)
  {
          p[i].r = p[i].g = p[i].b = i;
  }
  */
   set_palette(p);

  clear_to_color(buffer,  makecol(255,255,255));
  clear_to_color(screen,  makecol(255,255,255));
  //read_data();
```

```
   for(i = 0; i < SIZE; i++)
     for(j = 0; j < SIZE; j++)
              the_data[i][j] = 0;

sprintf(buf, "%s.txt", argv[l]);
fp = fopen(buf, V);
   while(get_line(buf, 80, fp))
   {
            sscanf(buf, "%s %s", var_buf_l, var_buf_2);
            x = atof (var_buf_l);
            y = atof(var_buf_2);
            nx=(int)x/(int)(400/SIZE);
            ny=(int)y/(int)(400/SIZE);
            the_data[nx] [ny]++;
            if (the_data[nx] [ny] > the_max) the_max = the_data[nx] [ny] ;
            if(nxx > 0 && nyy > 0) line(buffer,x,y,nxx,nyy,makecol(0,0,0));
            nxx=(int)x;
            nyy=(int)y;
   }
   fclose(fp);

   fp = fopenCoutput.txt", "w");
   for(i = 0; i < SIZE; i++)
   {
    for(j = 0; j < SIZE; j++)
    {

      intense = (int)(255 - (255 * (float) ((float)the_data[i][j]/(float)the_max)));
            if (intense < 0) intense = 0;
            fprintf(fp, "%d ", intense);
   /* rectf ill (buffer, i * (int) (400/SIZE),
                     j * (int)(400/SIZE),
                     i * (int)(400/SIZE) + (int)(400/SIZE),
                     j * (int)(400/SIZE) + (int)(400/SIZE),
                     makecol(intense, intense, intense));
   */
   /*       the_time= (float)the_data[i][J]/60;
            if((the_time > 0) && (the_time < 10))
                     circle (buffer,
                     i * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                     j * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                     0,
                     makecol (200,200,200));
            if((the_time >= 10) && (the_time < 30))
                     circle (buffer,
```

```
                        i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        3,
                        makecol (200,200,200));
                if((the_time >= 30) && (the_time < 120))
                        circle (buffer,
                        i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        10,
                        makecol(200,200,200));
                if((the_time >= 120) && (thejime < 300))
                        circle (buffer,
                        i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        15,
                        makecol(200,200,200));
                if (the_time >= 300)
                        circle (buffer,
                        i * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                        20,
                        makecol(200,200,200));

        */
        //        if((i>l)&(j>D)
        //                line   (buffer,the_data[i-l][j-l],the_data[i][j],makecol(200,200,200);


                }
          fprintf(fp,  "\n");
         }
         fprintf(fp,  "%d", the_max);
        fclose(fp);
        //circle(buffer, 235, 135, 130, makecol(200,200,200));
 get_palette(pal);
 sprintf(buf,  "%s.bmp", argv[l]);
 save_bitmap(buf,  buffer,  pal);
  blit(buffer, screen, 0, 0, 0, 0, buffer->w, buffer->h);
  textout_centre(screen, font, "Test", 600, 500, 0);
  //get_palette (tpal) ;
  //bmp = create_sub_bitmap(screen, 0, 0, SCREEN_W, SCREEN_H);

//while(!(key[KEY_ESC])) {};
 //get_palette(pal);
 //save_bmp("test.bmp", buffer, pal);
```

```
allegro_exit();

return 0;
 }

END_OF_MAIN();
```

///. *C++ Program written for Dispersion*

```
#include <stdlib.h>
#include <stdio.h>
#include <math.h>
#include <time.h>

#include "allegro.h"

#define SIZE 80
#define DISPCRIT 10
#def ine X 0
#define Y 1
#def ine D 2
#define SAMPLING 20
#define MAXPOINTS 20000
#def ine F 0
#def ine T 1
#def ine FAC 1

#define DEBUG T

#defineM_PI 3.141952

/* convert radians to degrees */
#define DEG(n)    ((n) * 180.0 / M_PI)

#define HEADCRIT 1
#define LENCRIT 1

int COUNT_disp = 0;
int the_data[SIZE] [SIZE];
int line_data[MAXPOINTS][2];
floatdisp_data[100][3];
int i;

float dispersion = 0;

BITMAP* buffer;
        FILE *fp;
        char buf [80];
        char var_buf_l[80], var_buf_2[80];
        float x, y;
        intnx, ny, j;
```

```
        int the_max = 0;
int COUNTcircles = 0;

int circles[3] [SIZE*SIZE];


float get_distance(float x1, float y1, float x2, float y2)
{
  float value;
  value = sqrt(pow(x2 - x1, 2) + pow(y2 - y1, 2));
  return value;
}

double headingerror(float x2, float y2, float x1, float y1, float x3, float y3)
{
  double he = 0.;
  float a, b, c;

  a = sqrt((pow((x2 - x3), 2)) + (pow((y2 - y3), 2)));
  b = sqrt((pow((xl - x3), 2) + pow((yl - y3), 2)));
  c = sqrt((pow((xl - x2), 2) + pow((yl - y2), 2)));

  he = ((-(pow(a, 2)) + (pow(b, 2)) + (pow(c, 2)))/ + (2 * b * c));
  he = acos(he);
  he = DEG(he);

  return he;
}


[illegible]

char *get_line(char *string, int maxLength, FILE *fp)
{
char buffer[512]; /* temporary string storage */

int length,     /* length of line read */
    index=0,    /* looping variables */
    index_2=0,
    parsed=0;   /* has the current input line been parsed */

/* get the next line of input, make sure there is something on the line */

while(l)
     {
     if   (!fgets(buffer,maxLength,fp))
```

```
      return (NULL);
length = strlen (buffer);

/* kill the carriage return */

buffer [length-1] = 0;

/* reset index */

index = 0;

/* eat leading white space */

while (buffer [index] ==' ')
        index++;

/* read line into buffer, if "#" arrives in data stream then disregard
 rest of line */

parsed=0;
index_2=0;

while(!parsed)
        {

        if  (buffer[index] !='#')
           {
           /* insert character into output string */

           string[index_2] = buffer [index];

           /* test if this is a null terminator */

           if  (string[index_2]==0)
                 parsed=l;

           /* move to next character */

           index++;
           index_2++;

           }
        else
           {
           /* insert a null termination since this is the end of the string */
           string [index_2] = 0;
```

```
            parsed=l;
            }
          }
          /* make sure there's a string there and not a blank line */
          if (strlen(string))
            return (string);

    }
}


int main(int argc, char **argv[])
{
 FILE *fp_disp;

 FILE *fp;
 PALETTE p;
 RGB temp;
 BITMAP *bmp;
 PALETTE tpal;
 PALETTE pal;
 int intense;
 float the_time;
 float tempf;

 charbuf[80];
 float he = 0;
int BOOLdraw_line = F;
 int lowbound = 0;
 int highbound = 0;
 int COUNTdata = 0;
 float sx, sy;
 float dist;
  intk;

float fx, fy;
float x2, y2;
float x3, y3;
float ex,ey;
int indl, ind2;


 allegro_init();
 install_keyboard();
 install_timer();
```

```
install_mouse();



set_color_depth(l 6);
set_gfx_mode(GFX_AUTODETECT_FULLSCREEN, 800, 600, 0, 0);
buffer = create_bitmap(400, 400);
//set_palette (desktop_palette);
generate_332_palette(p);
/*
  for(i = 0; i < 256; i++)
{
         p[i].r = p[i].g = p[i].b = i;
}
*/
 set_palette(p);

clear_to_color(buffer,  makecol(255,255,255));
clear_to_color(screen,  makecol(255,255,255));
//read_data();
        for(i = 0; i < SIZE; i++)
          for(j = 0; j < SIZE; j++)
                  the_data[i][j]  =0;
        for(i = 0; i < 100; i++)
       for(j = 0; j < 2; j++)
             disp_data[i] [j] = 0;

     sprintf(buf,  "%s.txt",  argv[l]);
        if(DEBUG)
        {
                printf("Filename buffer filled - %s\n", buf);
        }

     if(!(fp  =  fopen(buf,  "r")))
        {
                allegro_exit();
                printf ("Can't open data file\n");
                exit(0);
        }
        if(DEBUG)
        {
                printf ("File opened for reading - %s\n", buf);
        }
        while(get_line(buf,  80,  fp))
        {
```

```
        sscanf(buf, "%s %s", var_buf_l, var_buf_2);
        x = atof(var_buf_l);
        y = atof(var_buf_2);
        nx = (int)x/(int) (400/SIZE);
        ny = (int) y/(int) (400/SIZE);
        the_data[nx] [ny]++;
        if (the_data[nx] [ny] > the_max) the_max = the_data[nx][ny];
}
if (DEBUG)
{
        printf("Data read into arrays\n");


fclose(fp);


fp = fopenCoutput.txt", "w");
for(i = 0; i < SIZE; i++)
{
 for(j = 0; j < SIZE; j++)
 {

   intense = (int) (255 - (255 * (float) ((float)the_data[i][j]/(float)the_max)));
        if (intense < 0) intense = 0;
        fprintf(fp, "%d ", intense);
/* rectf ill (buffer, i * (int) (400/SIZE),
                j * (int)(400/SIZE),
                i * (int)(400/SIZE) + (int)(400/SIZE),
                j * (int)(400/SIZE) + (int)(400/SIZE),
                makecol(intense, intense, intense));
*/
        the_time = (float)the_data[i][j]/60;
   if (the_time >= DISPCRIT)
   {
     disp_data[COUNT_disp][0] = (int)i * (int) (400/SIZE) + (int)(400/SIZE)*5;
     disp_data[COUNT_disp][l] = (int)j * (int)(400/SIZE) + (int)(400/SIZE)*.5;
     disp_data[COUNT_disp][2] = the_time;
     COUNT_disp++;

   }

        if((the_time > 0) && (the_time < 10))
                circle (buffer,
                i * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                j * (int)(400/SIZE) + (int)(400/SIZE)*.5,
                0,
```

```
                makecol(200,200,200));
        if((the_time >= 10) && (the_time < 30))
                circle (buffer,
                i * (int) (400/SIZE) + (int) (400/SIZE)*.5,
                j * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                3,
                makecol (200,200,200));
        if((the_time >= 30) && (the_time < 120))
                circle (buffer,
                i * (int) (400/SIZE) + (int)(400/SIZE)*5,
                j * (int) (400/SIZE) + (int)(400/SIZE)*.5,
                10,
                makecol (200,200,200));
        if((the_time >= 120) && (the_time < 300))
    {
        circle (buffer,
                i * (int) (400/SIZE) + (int) (400/SIZE)*.5,
                j * (int) (400/SIZE) + (int) (400/SIZE)* 5,
                15,
                makecol(200,200,200));
    }
    if (the_time >= 300)
    {
        circles[X] [COUNTcircles] = x;//,i * (int) (400/SIZE) + (int)(400/SIZE)*.5;
        circles[Y] [COUNTcircles] = y;//j * (int) (400/SIZE) + (int)(400/SIZE)*.5;
                circles [D] [COUNTcircles] = 20;
                COUNTcircles++;
                circle (buffer,
                i * (int) (400/SIZE) + (int) (400/SIZE)* 5,
                j * (int) (400/SIZE) + (int) (400/SIZE)*.5,
                20,
                makecol(200,200,200));
    }

  }
  fprintf(fp,  "\n");
}
fprintf(fp,  "%d",  the_max);
fclose(fp);

if (DEBUG)
{
        printf("Data binnedVn");
}

//circle(buffer, 235, 135, 130, makecol(200,200,200));
```

```
for(i = 0; i < MAXPOINTS; i++)
  for(j = 0; j < 2; j++)
        line_data[i][j] = 0;

sprintf(buf, "%s.txt", argv[l]);
fp = fopen(buf, "r");
COUNTdata = 0;
i =0;
   while(get_line(buf, 80, fp))
{
      sscanf(buf, "%s %s", var_buf_l, var_buf_2);
            x = atof(var_buf_l);
            y = atof(var_buf_2);
      i++;
      if((i % SAMPLING) == 0)
      {
            line_data[COUNTdata][X] = x;
            line_data[COUNTdata][Y] = y;
            COUNTdata++;
      }
   }
   fclose(fp);

   fp = fopenCoutput.txt", "w");
for(i = 0; i < COUNTdata-3; i++)
{
      x2 = line_data[i+l][X];
      y2 = line_data[i+l][Y];
      x3 = line_data[i+2][X];
      y3 = line_data[i+2][Y];
      ind2 = i+2;

      if (i == 0)
      {
         indl = 0;
         ind2 = 2;
         f x = line_data[i] [X];
         fy = line_data[i][Y];
         sx = fx;
         sy = fx;
      }
     he = headingerror(fx, fy, x2, y2, x3, y3);
     if (he > HEADCRIT)
     {
      sx = fx;
```

```
            sy = fy;
            dist = get_distance(fx, fy, x3, y3);
            if (dist > LENCRIT)
            {
             fprintf (fp, "%.2f %.2f - %.2f %.2f - %.2f\n", fx, fy, x3, y3, dist);
            /* lineflbuffer, fx, fy,
                        x3, y3,
                        makecol (200,0,0));
              */


           BOOLdrawJine = F;
                  for(k = 0; k < COUNTcircles; k++)
                        {
                        tempf = get_distance(line_data[ind2][X], line_data[ind2][Y],
circles[X][k],circles[Y][k]);
                        if (tempf <= (circles [D][k] * FAC)) BOOLdrawJine = T;
                        fprintf (fp, "K = %d, %.2f\n", k, tempf);
                           }
                  if (BOOLdrawJine == T)
                  {
                           circlef ill (buffer,
                  line_data[indl][X],
                  line_data[indl][Y],
                  2,
                  makecol (100,100,100));

                for(j = indl; j < ind2 - 1; j++)
                {

                line(buffer, line_data[j][X], line_data[j][Y],
                        line_data[j+l] [X], line_data[j+l] [Y],
                        makecol (50,50,50));


                }
              }
                     }
              indl  =i+l;
              fx = x2;
              fy = y2;

             }
       }
  fclose(fp);
```

```
        if(DEBUG)
        {
                printf ("Buffer drawn\n");
        }




get_palette(pal);
sprintf(buf, "%s.bmp", argv[l]);
save_bitmap(buf, buffer, pal);

        if(DEBUG)
        {
                printf ("Bitmap saved\n");
        }

 blit(buffer, screen, 0, 0, 0, 0, buffer->w, buffer->h);
//  textout_centre(screen, font, "Test", 600, 500, 0);
 //get_palette (tpal);
 //bmp = create_sub_bitmap(screen, 0, 0, SCREEN_W, SCREEN_H);

while(!(key[KEY_ESC])) {};
//get_palette(pal);
//save_bmp("test.bmp", buffer, pal);


 sprintf(buf, "%s_disp.txt", argv[l]);
 fp_disp= fopen(buf, "w");

 for(i = 0; i < COUNT_disp; i++)
 {
     for(j = 0; j < COUNT_disp; j++)
     {
       if(i!=j)
         dispersion +=
           get_distance((float)disp_data[i] [0],
                   (float)disp_data[i][l],
                   (float)disp_data[j][0],
                   (float)disp_data[j][l]);

     }
```

```
 }
 dispersion = dispersion/(float)(COUNT_disp-l);
 dispersion = dispersion/(float)(COUNT_disp-l);
 fprintf(fp_disp, "DISPERSION %.2f for %d points\n", dispersion, COUNT_disp);
 for(i = 0; i < COUNT_disp; i++)
 {  fprintf(fp_disp,  "%.2At%.2f\tTIME:%.2f\n",
            disp_data[i][0],
            disp_data[i][l],
            disp_data[i][2]);

 }

 fclose(fp_disp);
 allegro_exit();

 return 0;
}

END_OF_MAIN();
```

IV. C++ Program written for Straight Lines/Direct Trips

```
#include <stdlib.h>
#include <stdio.h>
#include <math.h>
#include <time.h>

#include "allegro.h"

#define SIZE 80
#define SAMPLING 20
#def ine X 0
#def ine Y 1
#define MAXPOINTS 20000

#defineM_PI  3.141952

/* convert radians to degrees */
#define DEG(n)    ((n) * 180.0 / M_PI)

#define HEADCRIT 10
#define LENCRIT 60

int the_data[MAXPOINTS][2];
int i;
  BITMAP* buffer;
        FILE *fp;
        charbuf[80];
        char var_buf_l[80], var_buf_2[80];
        float x, y;
        intnx, ny,j;
        int the_max = 0;

float get_distance(float x1, float y1, float x2, float y2)
{
  float value;
  value = sqrt(pow(x2 - x1, 2) + pow(y2 - y1, 2));
  return value;
}

double headingerror(float x2, float y2, float x1, float y1, float x3, float y3)
{
  double he = 0.;
  float a, b, c;
```

```
  a = sqrt((pow((x2 - x3), 2)) + (pow((y2 - y3), 2)));
  b = sqrt((pow((xl - x3), 2) + pow((yl - y3), 2)));
  c = sqrt((pow((xl - x2), 2) + pow((yl - y2), 2)));

  he = ((-(pow(a, 2)) + (pow(b, 2)) + (pow(c, 2)))/ + (2 * b * c));
  he = acos(he);
  he = DEG(he);

  return he;
}
```

/********pt ^j> ^* *|> ^^ ^> ^^ ^^ ^^ »^p> ?% ^^ ?p> ^fh ^^ ^S ?pt ^|h ^pi ?|h *^> *]^ ^^ *^ ^|> '^ #% *^ *^ *^ *^h <|S *^ *T> ^^ *^ ^^ *^ ^* *^ ^^ ^^ *^ *^ ^^ 'p ^^ ^^ #^ »'S »^ #'h *^ ^^ »'S »'S *^ *^ *^h »'h »^ *^ »^ fi *f* *"|* *J* *y*

```
char *get_line(char *string, int maxLength, FILE *fp)
{
char buffer[512]; /* temporary string storage */

int length,      /* length of line read */
   index=0,     /* looping variables */
   index_2=0,
   parsed=0;    /* has the current input line been parsed */

/* get the next line of input, make sure there is something on the line */

while(l)
    {
    if   (!fgets(buffer,maxLength,fp))
         return (NULL);
    length = strlen (buffer);

    /* kill the carriage return */

    buffer[length-l] = 0;

    /* reset index */

    index = 0;

    /* eat leading white space */

    while(buffer[index]==")
            index++;

    /* read line into buffer, if "#" arrives in data stream then disregard
```

```
     rest of line */

    parsed=0;
    index_2=0;

    while(lparsed)
          {

          if (buffer[index] !=*#)
            {
            /* insert character into output string */

            string [index_2] = buff er [index];

            /* test if this is a null terminator */

            if (string [index_2]==0)
                  parsed=l;

            /* move to next character */

            index++;
            index_2++;

            }
          else
            {
            /* insert a null termination since this is the end of the string */
            string [index_2] = 0;
            parsed=l;
            }
          }
          /* make sure there's a string there and not a blank line */
          if (strlen(string))
            return (string);

    }
}



int main(int argc, char **argv[])
{

  FILE *fp;
  PALETTE p;
```

```
RGB temp;
BITMAP *bmp;
PALETTE tpal;
PALETTE pal;
int intense;
float he = 0;
float the_time;
int lowbound = 0;
int highbound = 0;
int COUNTdata = 0;
float sx, sy;
float dist;

charbuf[80];
float fx, fy;
float x2, y2;
float x3, y3;
float ex,ey;
int indl, ind2;

allegro_init();
install_keyboard ();
install_timer();
install_mouse();


set_color_depth(l 6);
set_gfx_mode(GFX_AUTODETECT, 800, 600, 0, 0);
buffer = create_bitmap(400, 400);
generate_332_palette (p);
  set_palette(p);

clear_to_color(buffer,  makecol(255,255,255));
clear_to_color(screen,  makecol(255,255,255));

    for(i = 0; i < MAXPOINTS; i++)
     for(j  =  0;j<2;j++)
                the_data[i][j] = 0;

    sprintf(buf,  "%s.txt",  argv[l]);
    fp = fopen(buf, V);
    COUNTdata = 0;
    i  =0;
       while(get_line(buf,  80,  fp))
    {
          sscanf(buf,  "%s %s", var_buf_l, var_buf_2);
```

```
            x = atof(var_buf_l);
            y = atof(var_buf_2);
        i++;
        if ((i % SAMPLING) == 0)
        {
            the_data[COUNTdata][X] = x;
            the_data[COUNTdata][Y] = y;
            COUNTdata++;
        }
    }

    fclose(fp);

    fp = fopenCoutput.txt", "w");
for(i = 0; i < COUNTdata-3; i++)
{
        x2 = the_data[i+l][X];
        y2 = the_data[i+l][Y];
        x3 = the_data[i+2][X];
        y3 = the_data[i+2][Y];
        ind2 = i+2;

        if (i == 0)
        {
           indl = 0;
           ind2 = 2;
           fx = the_data[i][X];
           fy = the_data[i][Y];
           sx = fx;
           sy = fx;
        }
       he = headingerror(fx, fy, x2, y2, x3, y3);
       if (he > HEADCRIT)
       {
        sx = fx;
        sy = fy;
        dist = get_distance(fx, fy, x3, y3);
        if (dist > LENCRIT)
        {
         fprintf(fp, "%.2f %.2f - %.2f %.2f -- %.2f\n", fx, fy, x3, y3, dist);
        /* line(buffer, fx, fy,
                    x3, y3,
                    makecol (200,0,0));
          */

           circlef ill (buffer,
```

```
                the_data[indl][X],
                the_data[indl][Y],
                2,
                makecol(100,100,100));

              for(j = indl; j < ind2 - 1 ; j++)
              {

               line (buffer, the_data[j][X], the_data[j][Y],
                        the_data[j+l][X], the_data[j+l][Y],
                        makecol (50,50,50));


              }
             }
             indl = i+l;
             fx = x2;
             fy = y2;

            }
       }
 fclose(fp);



 blit(buffer, screen, 0, 0, 0, 0, buffer->w, buffer->h);
              while(!(key[KEY_ESC])) {}

 //circle(buffer, 235, 135, 130, makecol(200,200,200));
 get_palette(pal);
 sprintfCbuf, "LINE%s.bmp", argv[l]);
 save_bitmap(buf, buffer, pal);

//  textout_centre(screen, font, "Test", 600, 500, 0);

 allegro_exit();

 printf("%.2f\n", headingerror(l, 0, 0, 0, 0, 1));

 return 0;
}

END_OF_MAIN();
```